

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

Annual Scientific and Technical Report

October 1, 2003 – September 30, 2004



Cooperative Agreement NCC 9-58

with the

**National Aeronautics and Space Administration
Lyndon B. Johnson Space Center
Houston, Texas**

September 30, 2004

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1.0 Introduction

This report outlines the National Space Biomedical Research Institute's (NSBRI) activities during FY 2004, the Institute's seventh year. It is prepared in accordance with Cooperative Agreement NCC 9-58 between NASA's Lyndon B. Johnson Space Center (JSC) and the Institute's lead institution, Baylor College of Medicine.

2.0 Background

In June 1996, NASA released a Cooperative Agreement Notice (CAN) inviting proposals to establish a National Space Biomedical Research Institute (9-CAN-96-01). This CAN stated that:

The Mission of the Institute will be to lead a National effort for accomplishing the integrated, critical path, biomedical research necessary to support the long term human presence, development, and exploration of space and to enhance life on Earth by applying the resultant advances in human knowledge and technology acquired through living and working in space. The Institute will be the focal point of NASA sponsored space biomedical research.

NASA has not amended this statement, and it remains the mission of the NSBRI.

The Institute was selected by NASA in March 1997 following a two-phase, competitive review of proposals received in response to the CAN. In April 1997, NSBRI was chartered in the State of Texas as a non-profit corporation. After a 60-day definition period, NASA and NSBRI signed a Cooperative Agreement (NCC 9-58) and Cooperative Agreement Management Plan (CAMP) with JSC. The Cooperative Agreement is for a five and one-half year base period and three, five-year optional extensions. The first optional extension, lasting until September 30, 2007, is being exercised. The CAMP was revised in June 2003 to fully align it with the Institute's Revised Strategic Plan and NASA's Bioastronautics Strategy, both approved in 2003.

NSBRI partners with NASA to develop countermeasures against the deleterious effects of long-duration space flight, and performs fundamental and applied space biomedical research directed toward this goal. This is accomplished by:

- designing, testing and validating effective countermeasures to address the biological and environmental impediments to long-term human space flight;
- defining the molecular, cellular, organ-level, integrated responses and mechanistic relationships that ultimately determine these impediments, where such activity fosters the development of novel countermeasures;
- establishing biomedical support technologies to maximize human performance in space, reduce biomedical hazards to an acceptable level and deliver quality medical care;
- transferring and disseminating the biomedical advances in knowledge and technology acquired through living and working in space to the general benefit of humankind; including the treatment of patients suffering from gravity- and radiation-related conditions on Earth; and

- ensuring open involvement of the scientific community, industry and the public in the Institute's activities and fostering a robust collaboration with NASA, particularly through JSC.

NSBRI is governed by a consortium of 12 institutions – Baylor College of Medicine, Brookhaven National Laboratory, Harvard Medical School, The Johns Hopkins University, Massachusetts Institute of Technology, Morehouse School of Medicine, Mount Sinai School of Medicine, Rice University, Texas A&M University, University of Arkansas for Medical Sciences, University of Pennsylvania Health System and University of Washington. The Institute's headquarters is in Houston at Baylor College of Medicine.

The Institute's initial peer-reviewed research program consisted of eight research teams carrying out 37, three-year projects and four, one-year synergy projects designed to bridge between discipline research team activities and create an atmosphere for future interdisciplinary research. After two open research announcements in FY 2000, the Institute expanded to 12 research teams and 85 research projects during its fourth year. In the fifth and sixth years, the number of research teams was consolidated to 11 and an integrated NSBRI/NASA space medicine program was added, bringing the total number of research projects to 94. In the seventh year, many projects completed their third and final year. An open solicitation in collaboration with NASA resulted in 48 new or competitively renewed projects, bringing the research project total to 80 at year end.

In addition to its research program, NSBRI developed vital education, outreach and communications programs that take advantage of the Institute's core research activities. Currently, research and education projects take place at more than 70 institutions across 21 states.

The Institute's management plan is based on the models used by the National Institutes of Health and Department of Defense. An independent Board of Scientific Counselors (BSC) is responsible for assuring excellence in the Institute's science and technology program through independent, external peer review. An External Advisory Council (EAC) provides advice to Institute management concerning programmatic relevance and effectiveness. NSBRI also has a User Panel of former and current astronauts and flight surgeons responsible for assuring the research program focuses on astronaut health and safety. An Industry Forum of representatives from aerospace, biomedical and technology industries assists in developing industry participation in NSBRI and in timely technology transfer.

Primary support for NSBRI's activities is furnished by NASA through NCC 9-58, although funds to support Institute activities also come from several sources, including the institutions involved in carrying out NSBRI's programs. Initial annual base funding for the Institute's first two years of operation (FY 1998 and 1999) was approximately \$10 million. In FY 2000, base annual funding increased to approximately \$14 million to develop the infrastructure needed to support planned program growth in FY 2001. In support of the expanded research and education programs, the base annual funding increased to approximately \$25 million in FY 2001 and was \$22 million in FY 2002. Budget stability of \$30 million per year for FY 2003-2007 was achieved. This figure is divided into \$29 million for core research, education and administrative costs and \$1 million for core space medicine activities. The Institute also supports \$2 to \$3 million of non-core research that funds a translational workforce and integration effort that helps link NSBRI and JSC programs. Approximately 85 percent of NSBRI costs are expended on research and development, 7 percent on education and public outreach, and 8 percent on headquarters administration.

3.0 Summary Table of FY 2004 Activities

Table 1. Major NSBRI Activities October 1, 2003 – September 30, 2004		
DATE	ACTIVITY	LOCATION
October 28	Behavioral Health Liaison Hired	N/A
November	Executive Science and Medicine Council Established	N/A
November 14	NSBRI/NASA Education and Public Outreach Planning Meeting	Houston
November 15	Peer Review of NRA 03-OBPR-04 Proposals Ends	Washington, DC
December 1	Associate Director Appointed	N/A
December 10	External Advisory Council Executive Session	San Diego
December 16	NRA-03-OBPR-04 Selection Meeting for Team Leader Projects	Washington, DC
January 5-10	NSBRI Education and Public Outreach Team attend OBPR Education/Outreach Annual Conference	New Orleans
January 12-15	NSBRI Investigators' Retreat	Montgomery, TX
January 30	NSBRI/NASA Space and Life Sciences Directorate Senior Management Meeting	Seabrook, TX
February 10-12	External Advisory Council Meeting	Houston
February 15	Summer Internship Applications Due	N/A
March 4	Selection Meeting for NRA-03-OBPR-04	Washington, DC
March 19	Industry Forum Teleconference	N/A
March 25	Board of Directors Meeting	Houston
April 8	Summer Internship Selection Letters Distributed	N/A
April 14	Postdoctoral Fellowship Program Request for Proposals Released (NSBRI-RFP-04-01)	N/A
May	NSBRI/NASA Space Radiation Liaison Appointed	N/A
May 24	Education and Public Outreach Request for Proposals Released (NSBRI-RFP-04-02)	N/A
June 4	NASA/NSBRI NNH04ZUU003N Released	N/A
June 30	Postdoctoral Fellowship Program Applications Due	N/A
July 28	Education and Public Outreach Proposals Due	N/A
August 23-24	Review of Postdoctoral Fellowship Applications	New York
August 26-27	Peer Review of Education and Outreach Proposals	Houston
September 8	NNH04ZUU003N Proposals Due	N/A
September 22-23	External Advisory Council Meeting	Houston

4.0 Strategic Directions

In FY 2003, NSBRI's Strategic Plan was revised and updated following a successful review of the Institute's activities and progress wherein it was noted that "The benefits of the NSBRI Program to NASA and to health care on earth are potentially very significant. NASA must therefore commit to adequate and stable funding for the Institute to be able to develop effective countermeasures to enhance safety of prolonged and recurrent space travel." As outlined in the Revised Strategic Plan, and in accordance with the Vision for Space Exploration announced on January 14, 2004, the Institute has effectively implemented the stated Aims and Objectives, as outlined in this section.

As stated in the Revised Strategic Plan, NSBRI is tasked with integrating the knowledge base relevant to the biomedical response of humans in space, understanding and quantifying the risk levels associated with this knowledge base, and recommending acceptable risk levels for long-duration missions. NSBRI has maintained an integral role in support of revision and refinement of the Bioastronautics Roadmap, which delineates risks according to health category. The Director and Associate Director serve as discipline leads for targeted risk areas on the Bioastronautics Science Management Team. In accordance with the revised Roadmap, NSBRI has developed a countermeasure product pipeline, identifying risk areas addressed by specific research projects and tracking Institute investment in scientific and technology countermeasure research and development as a function of NASA's Human Health and Performance product lines. The revised Roadmap will be presented to the Institute of Medicine in November 2004.

NSBRI has developed, managed and implemented an integrated research plan that is developing the required knowledge and technologies to enable long-duration space flight, and implements a "best value" research program approach, a stated objective of the Revised Strategic Plan. To ensure alignment of the research program with the Aims and Objectives of the Institute, a Programmatic Relevance Assessment metric was developed (Appendix A), with input from NSBRI's BSC and EAC. The assessment, also vetted through NASA, is based upon strategic need, product development and return on investment. Using this metric, new research projects were selected in response to NRA 03-OBPR-04 (Appendices H and I, and Section 5.1.a and 5.1.b.). An efficient electronic proposal submission and project management system is operational, thereby facilitating streamlined bookkeeping capabilities and enhanced access to research project detailed information.

In alignment with the Revised Strategic Plan's objectives, NSBRI made significant progress this year facilitating an understanding of, and promoting access to, the space medicine environment and the NASA space infrastructure associated with biomedical research through an integrated on-site presence at Johnson Space Center. A Memorandum of Understanding is in the final stages for establishment of an NSBRI Integration Facility, which will be developed in cooperation with NASA and Wyle Laboratories to facilitate integration of advanced technologies and medical capabilities to support exploration-class missions. A NASA/NSBRI joint working group for the Clinical Evaluation and Validation Project was also formed this year. The group evaluates progress and selections for flight studies as well as bed-rest analog projects.

Additionally, the past year was highlighted by optimized management practices. As stated in the Revised Strategic Plan, NSBRI provides a management process that supports the overall human in space biomedical research program. An expanded organizational structure (Table 2) was developed during the year, with the appointment of NSBRI/NASA Liaisons in the areas of Behavioral Health and Space Radiation (Section 7.1). Membership of the EAC and BSC was updated (Sections 7.3 and 7.4, respectively). The Executive Science and Medicine Council was formed, comprised of the Institute Director, Associate Director, Behavioral Health, Space

Medicine and Space Radiation Liaisons, and the Chief of Staff. This group meets on a weekly basis and oversees internal management optimization for refinement and prioritization of strategic needs and tactical implementation for exploration. The Council facilitates, and works in cooperation and coordination with, the Team Leaders, NSBRI Headquarters management and NASA.

Throughout the year, NSBRI management met with key NASA personnel to ensure maximum productivity and the Institute's alignment to overall goals and objectives. Daily contact between NSBRI and NASA was the norm, with several regularly scheduled tag-ups throughout the week. Strategic alignment of the Institute in accordance with the Vision for Space Exploration was outlined, and ongoing dialogue with senior NASA management was maintained via bimonthly NSBRI/NASA Steering Committee meetings. An NSBRI/NASA Space and Life Sciences Directorate Senior Management meeting was held on January 30. An Institute Status Report was presented to the Biological and Physical Research Advisory Committee at NASA Headquarters in May. The briefing detailed scientific and management achievements and plans for future growth in continued alignment with the Vision for Space Exploration.

Advances in knowledge resulting from NSBRI scientific programs and countermeasure research are communicated effectively to the community by means of an active Education and Public Outreach Program (Section 6.0). This program provides added value to NASA's education and outreach activities in several ways. NSBRI's Education and Public Outreach Program was expanded this year to include support for development of Phase I of a Graduate Education Program in Space Life Sciences, as well as support for four, two-year postdoctoral fellowships, which will be competed on an annual basis. This expansion provides a continuous educational pipeline from kindergarten through independent investigator, in accordance with the educational initiatives outlined in NASA's education strategy. Active collaboration with for-profit entities is maintained through NSBRI's Industry Forum (Section 8.1), with planned industry co-sponsorship of the newly-initiated Postdoctoral Fellowship Program.

5.0 Research Program

5.1 Scientific and Technical Core Research Program and Achievements

NSBRI's research program consists of investigator groups working on complementary projects focused on a common theme. Team management is the responsibility of a program director called a Team Leader, who works with the Associate Team Leader to prepare and implement a team strategic plan. Team and Associate Team Leaders participate in monthly telecons with the Director, Associate Director and other NSBRI management to coordinate activities among the teams and with NASA. Additional focused meetings within and between teams occur throughout the year to refine the peer-reviewed research program and ensure that NSBRI countermeasure development research is clearly focused on high-risk areas and important enabling questions on the Bioastronautics Roadmap. A complete listing of project executive summaries for NSBRI's core research program is contained in Appendix B. The Research Team Annual Reports for FY 2004 are in Appendix C.

The NSBRI Investigators' Retreat held in January included an interactive demonstration of countermeasure deliverables in development (Appendix D). Several demonstrations highlighted research and development occurring at the Countermeasure Readiness Level (CRL) 6-7, with near-term potential to transition to the Clinical Evaluation and Validation Project. More than 260 persons attended the Retreat, including NSBRI research team members and international partners, NASA investigators, Johnson Space Center Space and Life Sciences Directorate management, flight surgeons, astronauts, NASA Headquarters personnel, and members of

NSBRI's EAC and Industry Forum. During the Retreat, meetings were also held on refinement of the Bioastronautics Roadmap and the Technology Roadmap.

The Director's report presented at the March 2004 Board of Directors Meeting is included in Appendix E. In addition, a listing of NSBRI publications and presentations during FY 2004 appears in Appendix F.

Select research highlights include:

- Babs Soller, Ph.D., Associate Team Leader for Smart Medical Systems, was issued U.S. Patent No. 6,766,188 in July, entitled "Tissue Oxygen Measurement System," based upon her NSBRI-funded work.
- An invention disclosure based upon the NSBRI-funded work of Stephen Kosslyn, Ph.D., of the Neurobehavioral and Psychosocial Factors Team, was submitted entitled "A System and Method for On-Site Cognitive Efficiency Assessment." This work, aimed at developing a tool box for assessing the efficacy of specific aspects of cognitive processing, uses hand-held Palm Pilot technology.
- Scott Dulchavsky, M.D., of the Smart Medical Systems Team, moved his project on ultrasound for medical diagnosis and noninvasive imaging to flight. It is manifested on International Space Station Increments 8-11. Deliverables from this work to both NASA and Earth-based medicine include computer-based just-in-time ultrasound training products for non-medical personnel in remote environments, including in-flight scanning with remote guidance from ground experts.
- The NSBRI-funded neutron energy spectrometer developed by Richard Mauer, Ph.D., of the Technology Development Team, has been selected for incorporation into the Martian Radiation Environment Experiment on the Mars Odyssey mission.
- The miniature time-of-flight-mass spectrometer developed by Richard Potember, Ph.D., of the Technology Development Team, is under consideration by Space Medicine for real-time detection of analytes in complex biological fluids, air and water.
- The work of George Brainard, Ph.D., Associate Team Leader of Human Performance Factors, Sleep and Chronobiology, has shown that light treatment with prominent blue composition in the visible spectrum is a means to provide enhanced entrainment of sleep-wake patterns and circadian rhythms. This work has operational relevancy and is leveraged extensively with Philips Lighting.

A Supplemental Medical Objective has been developed by Jay Shapiro, M.D., of the Bone Loss Team, to evaluate the effectiveness of zoledronic acid, a long-lived bisphosphonate, for prevention of bone loss in flight. This countermeasure has transitioned from CRL 2-3 to 7 through NSBRI support. A recently-conducted randomized, double blind, placebo-controlled study in spinal cord injury patients, an analog model for microgravity exposure, demonstrated that a single intravenous injection with this agent afforded significantly less loss of bone mineral density, bone cross sectional area, periosteal diameter, section modulus, and average cortical thickness over a period of one year. This work represents a collaborative effort between NSBRI, NASA and Universities Space Research Association, and is being worked through the Countermeasure Evaluation and Validation Project.

A highly productive joint retreat of the Smart Medical Systems and Technology Development Teams was held in September. In addition to team member presentations, the retreat also featured an interactive workshop with flight surgeons, astronauts and representatives of Space Medicine from Johnson Space Center. Discussion focused on means of assessing operational relevance of developing countermeasures and technologies, and on mechanisms of transitioning this work to flight.

5.1.a. Team Leadership – NSBRI CFC-03-01

NSBRI received 21 applications for team leadership positions in response to an open Call for Candidates Soliciting Applications for Team Leadership (NSBRI CFC-03-01), released on April 15, 2003. The Policy on Team Leadership (Appendix G), which forms part of the NSBRI Revised Strategic Plan, was included in the Call for Candidates. Fourteen of these proposals had merit scores of 70 or greater and were subsequently assessed for cost and programmatic relevance.

The applications, along with accompanying research materials from the applicants, were discussed in detail during an EAC executive session held on December 10, 2003. The Selection Meeting for the Team Leader Projects occurred on December 16 in Washington (Appendix H). In January 2004, the Board of Directors approved new/competitively renewed Team Leader positions for:

Bone Loss	Peter Cavanagh, Ph.D., D.Sc.(Med.)	Cleveland Clinic
Cardiovascular Alterations	Richard Cohen, M.D., Ph.D.	MIT
Human Performance Factors, Sleep and Chronobiology	Charles Czeisler, Ph.D., M.D.	Harvard/Brigham & Women's Hospital
Immunology, Infection and Hematology	Ann Kennedy, D.Sc.	U. of Pennsylvania
Muscle Alterations and Atrophy	Kenneth Baldwin, Ph.D.	UC, Irvine
Neurobehavioral and Psychosocial Factors	David Dinges, Ph.D.	U. of Pennsylvania
Neurovestibular Adaptation	Charles Oman, Ph.D.	MIT
Smart Medical Systems	Lawrence Crum, Ph.D.	U. of Washington
Technology Development	Jay Buckey, Jr., M.D.	Dartmouth

Six of the nine individuals served as Team Leaders prior to the open competition (Drs. Cohen, Czeisler, Baldwin, Dinges, Oman and Crum [interim]). Two scientists were Associate Team Leaders who were promoted to Team Leaders (Drs. Kennedy and Buckey). Dr. Cavanagh, a new NSBRI investigator with NASA flight experiment experience, now serves as Team Leader for the Bone Loss Team.

After the other project proposals to NRA 03-OBPR-04 were reviewed, Associate Team Leaders were appointed as follows:

Bone Loss	Susan Bloomfield, Ph.D.	Texas A&M
Cardiovascular Alterations	Art Shoukas, Ph.D.	Hopkins
Human Performance Factors, Sleep and Chronobiology	George Brainard, Ph.D.	Thomas Jefferson University
Immunology, Infection and Hematology	Alan Gewirtz, M.D.	U. of Pennsylvania
Muscle Alterations and Atrophy	Alfred Goldberg, Ph.D.	Harvard
Neurobehavioral and Psychosocial Factors	Joseph Brady, Ph.D.	Hopkins
Neurovestibular Adaptation	Jacob Bloomberg, Ph.D.	NASA JSC
Nutrition, Physical Fitness and Rehabilitation	Vincent Caiozzo, Ph.D.	UC, Irvine
Smart Medical Systems	Babs Soller, Ph.D.	UMass Med School
Technology Development	Yi-Xian Qin, Ph.D.	Stony Brook – SUNY

5.1.b. Selection of NRA 03-OBPR-04

On April 15, 2003, NRA 03-OBPR-04 was released in conjunction with NASA soliciting Ground-Based Research Proposals for the Biomedical Research and Countermeasures Program. The announcement closed on July 15, 2003. NSBRI received 111 proposals to the call for proposals to one of ten NSBRI research teams. All proposals were peer reviewed, and peer review was completed in November 2003. Eighty proposals achieved a scientific merit score of 65 or greater and were evaluated for program relevance and cost. Nine NASA proposals with a scientific merit score were also considered by NSBRI.

Program relevance was assessed using the Relevance Assessment metric (Appendix A) by members of the NSBRI Executive Science and Medicine Council and by John Charles, Ph.D., representing NASA. The assessment of cost included the NSBRI Manager of Finance.

A final prioritization based upon merit, relevance and cost was vetted with the EAC and presented for joint selection with NASA Headquarters. The projects of team leadership candidates (Section 5.1.a) were selected through EAC and NASA Selection Meetings held in December. The remaining projects were discussed later with the new team leaders participating in the EAC meeting. For NRA 03-OBPR-04, ten team leader projects were selected followed by an additional 37 research proposals in March. One radiation technology proposal was brought before the NASA Radiation Board and subsequently selected by NSBRI in May. The average scientific merit score was 81 (out of a possible 100) for non-team leaders (for team leaders the average merit score was 88), with an average adjusted relevance score of 4.3, out of a possible 5.0. Category I proposals (relevance of 3.0-5.0, and excellent scientific merit of 90-100) comprised 41.7 percent of selected projects, 50 percent were Category II (relevance of 4.0-5.0, and very good scientific merit of 80-89), and 8.3 percent were Category III (relevance of 3.0-5.0 and good scientific merit of 70-79). During the evaluation process, specific consideration was given to the need to move projects to CRL 7 or beyond. The selection notes are Appendix I.

5.1c. Proposal from NRA 03-OBPR-06

One proposal selected by NASA was transferred to NSBRI, consisting of a NASA/ESA/CNES international long-term bed-rest study. The proposal was transferred to the Immunology, Infection and Hematology Team and will evaluate a variety of immune parameters and associated measures of stress, during extended bed rest.

5.1.d. Research Announcement NNH04ZUU003N

Following the precedent established in FY 2001, NSBRI and NASA collaborated on a joint solicitation covering Ground-Based Studies for Human Health in Space. The announcement, NNH04ZUU003N, was released on June 4, with proposals due on September 8. The call solicited proposals that address three distinct objectives: NSBRI Team Research, Individual Biomedical Model Systems Investigator Research, and Individual Biomedical Research and Countermeasures Investigator Research. The announcement was widely disseminated, including prominent displays on the NSBRI and NASA Web sites.

NNH04ZUU003N requested proposals to 10 NSBRI teams, which included all research teams but the Radiation Effects Team. NSBRI clearly emphasized the need for team-based countermeasure research and development that was translational and at the mid-range countermeasure readiness levels. All proposals were required to link research to the Bioastronautics Roadmap and its enabling questions. The NSBRI portion of the announcement focused on specific gaps in the current team programs.

Investigators proposing to the NSBRI portion of the announcement used NSBRI's electronic submission system, and it worked well. Submissions to NNH04ZUU003N will undergo peer review for scientific merit in FY 2005.

5.2 Core Space Medicine Program Achievements

FY 2004 was highlighted by significant progress in linking NSBRI science and technology advances with NASA space medicine. The Medical Operations Support Team, led by Hal Doerr, M.D., represents an integrated team with an operational and clinical focus. The project brings together NSBRI Investigators and operational personnel. The work focuses on training for medical emergencies in space using a patient simulator and involves debriefing following simulation in all aspects of team health care delivery, including physiology, pharmacology, behavioral, communications and operational protocol review. The work has been validated in KC135 flight testing, advancing the work to CRL 7. Deliverables include both skill-set training for individuals and team resource management/crisis resource management tools for the entire flight control team.

Scott Dulchavsky, M.D., moved his project on ultrasound for medical diagnosis and noninvasive imaging to flight, manifested on International Space Station increments 8-11. Babs Soller, Ph.D., Associate Team Leader for Smart Medical Systems, advanced her system and method for non-invasive blood and tissue measurement to Countermeasure Readiness Level/Technology Readiness Level (CRL/TRL) 6, and was successful in augmenting her NSBRI support for this project with a large grant from the Army Research Office. Lawrence Crum, Ph.D., Team Leader for Smart Medical Systems, continues to make marked advances in portable ultrasound technology for imaging and high-intensity focused ultrasound for treatment.

NSBRI has taken a strong position in the development of autonomous medical care for exploration. As NSBRI/NASA Space Medicine Liaison, Jonathan Clark, M.D., was effective in linking Team Leaders and Investigators to flight surgeons early in the R&D pipeline, as well as in instances where testing, evaluation and operational integration were relevant. NSBRI continued to participate in weekly Space Medicine activities at Johnson Space Center.

5.3 Partnerships: NASA, Other Institutions and International

Reformulation of the Bioastronautics Roadmap is ongoing to consolidate and reorganize risks and to better align the Roadmap with changing NASA Headquarters priorities, evolving exploration initiatives, and definition of mission scenario requirements. NSBRI has actively supported these revisions and has documented and restructured, where necessary, its activities in strong alignment with the Report of the President's Commission on Implementation of United States Space Exploration Policy: A Journey to Inspire, Innovate, and Discover. This Report, also known as the Aldridge Commission Report, makes recommendations that are consistent with how NSBRI provides unique capabilities to NASA. In implementing aspects of the Report through the creation of the Exploration Systems Mission Directorate, NSBRI has organized its science and technology portfolio along product lines and linked projects to the spiral development model.

NSBRI's Senior Management, Team Leadership, Principal Investigators, and Behavioral Health, Space Medicine, and Space Radiation Liaisons, all played a role in reorganization efforts of the Bioastronautics Roadmap and how it links to the Exploration Systems Mission Directorate. Jay Shapiro, M.D., member of the Bone Loss Team, presented a briefing on bone loss in space to the Institute of Medicine during the review of the Roadmap. This year, Chuck Oman, Ph.D., Neurovestibular Adaptation Team Leader, joined Kenneth Baldwin, Ph.D., Muscle Alterations and Atrophy Team Leader, as an appointed member of the Biological and Physical Research

Advisory Committee, which is currently in transformation given the establishment of the Exploration Systems Mission Directorate.

Throughout the year, NSBRI participated in numerous activities with NASA Centers, such as the annual meeting of the NASA Cell Science Investigators Working Group, the NASA Astrobiology Institute Executive Council Meeting, the Workshop on Animal Research in Support of Human Exploration, a NASA Headquarters Workshop on Requirements for Human Subjects in Exploration Research, and the NASA Bed Rest Project Investigator Working Group. The Associate Director was named to Board of Directors of BioServe Space Technologies, a NASA-sponsored Research Partnership Center. NSBRI initiated interactions and the review of potential collaborations between NSBRI and the National Center for Microgravity Research, associated with NASA Glenn Research Center. The Institute also prepared a Readiness for Flight briefing on NSBRI project status, which identified 11 studies with significant flight definition for operational capacity on International Space Station, five emerging but less operationally-mature supporting studies, and five enabling technologies with demonstrated proof-of-principle in ground-based testing.

NSBRI participated in several international activities during FY 2004. The Director, together with Drs. Jay Shapiro and Joseph Kerwin, attended a meeting with Novartis Pharmaceuticals in Basel, Switzerland. Presentations included the Supplemental Medical Objective on evaluation of zoledronate as a countermeasure for bone loss during extended space missions. NSBRI continues its efforts on the International Artificial Gravity Project, which is coordinated through NASA Headquarters, and participated in a review of the short-arm centrifuge. The Institute was well represented by Senior Management, Liaisons, Team Leaders and Principal Investigators at the Annual Meeting of the Society for Research on Biological Rhythms held in Vancouver, BC, at which an NSBRI-sponsored workshop was held on Circadian and Sleep Countermeasures for Space Exploration. During this workshop, George Brainard, Ph.D., presented an interactive demonstration of the blue light technology for circadian shifting.

6.0 Education and Public Outreach Program

In FY 2004, the NSBRI Education and Public Outreach Program underwent significant reorganization and expansion. The year began with an NSBRI/NASA Education and Public Outreach Planning Meeting to outline coordinated strategies on educational activities, with representation from Institute Senior Management, the NSBRI Education Team Leadership, NASA JSC Education Outreach and NASA Headquarters. The Team's Annual Report is included as Appendix J.

Several additional high-level meetings occurred during the year, including the team's participation in the Office of Biological and Physical Research's Education and Outreach Annual Conference. Members of the Education and Public Outreach Team participated extensively in the National Science Teachers Association convention, held in Atlanta. NSBRI educator resources were widely distributed at the event, and NSBRI Distinguished Educator Awards were presented to six nationally-recognized educators for their contributions to space life sciences education. Also at the event, Morehouse School of Medicine President, James Gavin III, M.D., Ph.D., presented the Morehouse School of Medicine Presidential Appreciation Award to NSBRI Director, Dr. Jeffrey Sutton.

Education and Public Outreach Team Leader, Marlene MacLeish, Ed.D., represented NSBRI at the NASA Office of Biological and Physical Research Scottish Enterprise Mission, which was held at University of Strathclyde. The event consisted of two weeks of teaching and classroom presentations, and reached more than 20,000 students from across Scotland. Dr. MacLeish also

leads an active after-school space club for underrepresented students, in conjunction with the Boys and Girls Clubs of Atlanta.

The NSBRI Food and Fitness Teachers Activity Guide, developed by NSBRI Education Team members from Baylor College of Medicine, was the basis for a NASA Headquarters Webcast on "Food for Space Explorers." This activity guide was also a major component of NASA Connect televised educational programming, produced by NASA Langley. The NASA Connect segment "Better Health from Space to Earth," featuring activities from NSBRI Teacher Activity Guides, was awarded a Silver Screen Award, the Best of Class COPPER AXIEM AWARD™ and was nominated for a regional Emmy Award in the category of Entertainment Programming. This endeavor was a joint collaboration between NSBRI, NASA Langley and Johnson Space Center.

NSBRI Education Team members are currently working with NASA on additional educational programming and are adapting the NSBRI "From Outer Space to Inner Space" teacher activity guides into at least four more television broadcasts that will also have supporting Web-based resources. NSBRI educational materials have also been used extensively in NASA SCI Files, a distance learning initiative for enhancing the teaching of math, science, geography and technology for grades three through five. Activities from NSBRI's Muscles and Bones Teacher Activity Guide are being used to develop the NASA Connect television program "Good Stress: Building Better Bones and Muscles." NASA Connect reaches grades six through eight.

The annual NSBRI Summer Internship Program was successful. Of the 25 applications received from students across the country, 13 were selected and placed in laboratories at Johnson Space Center. The internships ended with a banquet honoring the interns and their mentors; Dr. Adena Loston, NASA Headquarters Chief Education Officer, attended the event. The list of summer interns and their internship reports appear in Appendix K.

6.1 K-16 Program Announcement

A request for proposals for Expansion of Education and Public Outreach Activities, NSBRI-RFP-04-02, was released on May 24. The solicitation closed on July 28. In response to the K-16 portion of this RFP, 26 applications were peer-reviewed by an external panel. Following discussion in executive session with the EAC, recommendations were made to fund the four highest-scoring proposals, which ranged from 84 to 88 (out of a possible 100) in merit score. Three of the currently funded K-16 programs successfully re-competed their projects (Marlene MacLeish, Ed.D., Morehouse School of Medicine, William Thomson, Ph.D., Baylor College of Medicine, and Roland Smith, Ph.D., Rice University), and a new project (Gary Coulter, Ph.D., Colorado Consortium for Earth and Space Science Education) was also funded. The Selection Notes appear in Appendix L.

6.2 Graduate Education and Postdoctoral Programs

In addition to K-16 activities, the request for proposals for Expansion of Education and Public Outreach Activities, NSBRI-RFP-04-02, also solicited proposals for definition (Phase I) of a Graduate Education Program in Space Life Sciences. Six applications were received. Following peer review and discussion in executive session with the EAC, the recommendation was made to fund the two highest-scoring proposals (merit scores of 86 and 88, out of a possible 100). (Appendix L)

This year also marked the launch of NSBRI's Postdoctoral Fellowship Program. On April 14, NSBRI released an open solicitation, NSBRI-RFP-04-01, requesting proposals for postdoctoral fellowship applications. The RFP was advertised nationally (*Science*), and the solicitation closed on June 30. Twenty-eight applications were peer reviewed by the Postdoctoral Fellowship

Committee and scored according to scientific merit and program relevance, research background and qualifications of the candidate, and research mentor and environment. Final scores ranged from 60 to 94; four proposals were selected for funding (score range of 86 to 94). Fellows will be funded for a two-year period, and applications for the program will be solicited on an annual basis. Selection Notes appear in Appendix M.

7.0 Management

7.1 Organization and Key Personnel

The NSBRI Associate Director Search Committee conducted a national search and reviewed applications from many highly qualified individuals for the position of Associate Director. Promising candidates were interviewed by NSBRI and NASA personnel, and the Search Committee nominated Jeanne Becker, Ph.D., for the position. After her appointment was approved by NSBRI's Board of Directors, Dr. Becker assumed the position of Associate Director, effective December 1, 2003.

Two additional key personnel were hired during the year. Edna Fiedler, Ph.D., was hired as NSBRI/NASA Behavioral Health Liaison on October 28, 2003. Dr. Fiedler is instrumental in bridging the gap between NSBRI research and operations. She works closely with Team and Associate Team Leaders on the Human Performance Factors, Sleep and Chronobiology Team and the Neurobehavioral and Psychosocial Factors Team, as well as with the NASA Chief Psychiatrist. In May, Marcelo Vazquez, M.D., Ph.D., scientist in the medical department of Brookhaven National Laboratory, was appointed as the NSBRI/NASA Space Radiation Liaison. As Space Radiation Liaison, Dr. Vazquez will enhance the coordination of NSBRI's radiation activities in science, technology and education with NASA's Radiation Program, including the NASA Space Radiation Laboratory at Brookhaven.

As mentioned earlier, the Executive Science and Medicine Council was formed during FY 2004. This Council is comprised of the Institute Director, Associate Director, Behavioral Health, Space Medicine and Space Radiation Liaisons, and the Chief of Staff. The group meets on a weekly basis to monitor research progress and track compliance with Institute policy and procedures, as well as to prioritize strategic needs in alignment with national goals for space exploration.

The current NSBRI Organizational Chart appears in Table 2.

7.2 Board of Directors

The current NSBRI Board of Directors appears in Table 3. The Board met once in Houston (March 25) with a second meeting planned early in FY 2005 (Oct. 7). Dr. Frederick B. Rudolph, Rice University, died a few months after taking a position on the Board. He had strongly supported NSBRI since its inception.

During FY 2004, five new members joined, either filling a vacancy or replacing a member who stepped down. New members included Dennis S. Charney, M.D., Mount Sinai School of Medicine; Sandra A. Harris-Hooker, Ph.D., Morehouse School of Medicine; Eugene H. Levy, Ph.D., Rice University; Robert E. McGehee, Jr., Ph.D., University of Arkansas for Medical Sciences; and Larry D. Milne, Ph.D., University of Arkansas for Medical Sciences. Board members stepping down included Thomas E. Andreoli, M.D., University of Arkansas for Medical Sciences; E. Nigel Harris, M.D., Morehouse School of Medicine; Peter Paul, Ph.D., Brookhaven National Laboratory; Mary R. Rifkin, Ph.D., Mount Sinai School of Medicine; and I Dodd Wilson, M.D., University of Arkansas for Medical Sciences.

Table 2. NSBRI Organizational Structure

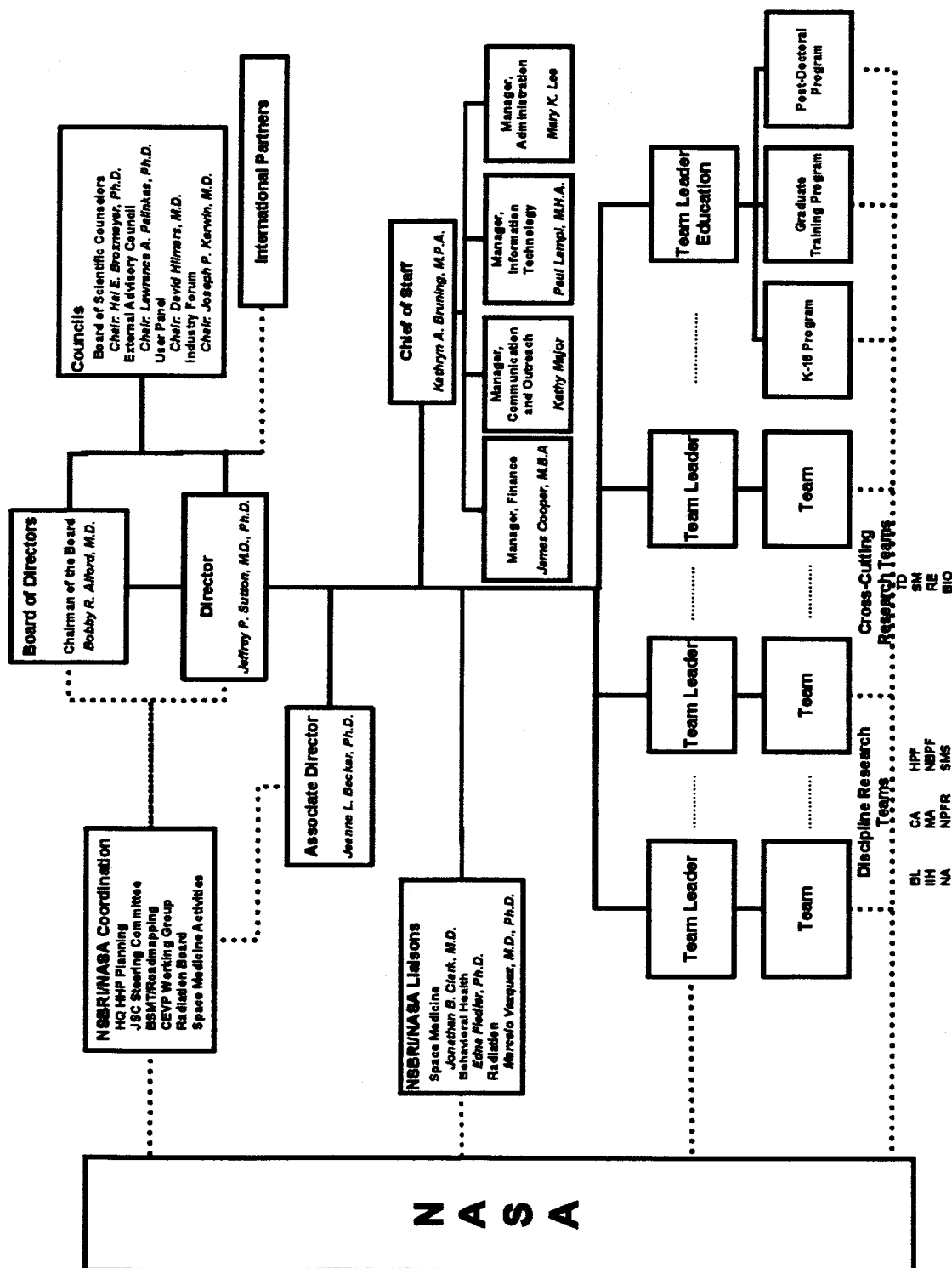


Table 3.**NSBRI Board of Directors**

Bobby R. Alford, M.D. (Chairman) Baylor College of Medicine	William L. Allen National Geographic Magazine	Carl W. Anderson, Ph.D. Brookhaven National Laboratory
James B. Bassingthwaight, M.D., Ph.D. University of Washington	Joseph V. Bonventre, M.D., Ph.D. Harvard-MIT Division of Health Sciences and Technology Harvard Medical School	James F. Buchli United Space Alliance
Dennis S. Charney, M.D. Mount Sinai School of Medicine	Michael E. DeBakey, M.D. Baylor College of Medicine	Richard E. Ewing, Ph.D. Texas A&M University
Alfred P. Fishman, M.D. University of Pennsylvania Health System	Theresa W. Fossum, D.V.M., Ph.D. Texas A&M University	Alice P. Gast, Ph.D. Massachusetts Institute of Technology
Glen N. Gaulton, Ph.D. University of Pennsylvania Health System	Martha L. Gray, Ph.D. Harvard-MIT Division of Health Sciences and Technology Massachusetts Institute of Technology	Sandra A. Harris-Hooker, Ph.D. Morehouse School of Medicine
Craig J. Hogan, Ph.D. University of Washington	Richard J. Johns, M.D. The Johns Hopkins University School of Medicine	Joseph P. Kerwin, M.D. Wyle Laboratories (ret.)
Steven Knapp, Ph.D. The Johns Hopkins University	Jordan Konisky, Ph.D. Rice University	Eugene H. Levy, Ph.D. Rice University
J. David Litster, Ph.D. (Emeritus) Massachusetts Institute of Technology	Robert E. McGehee, Jr., Ph.D. University of Arkansas for Medical Sciences	Larry D. Milne, Ph.D. University of Arkansas for Medical Sciences
Lawrence A. Palinkas, Ph.D. (<i>Ex Officio</i>) University of California, San Diego	James W. Patrick, Ph.D. Baylor College of Medicine	Hon. John E. Porter Hogan & Hartson
Alan L. Schiller, M.D. Mount Sinai School of Medicine	Kenneth I. Shine, M.D. The University of Texas System	Walter W. Sullivan, Ph.D. Morehouse School of Medicine
Jeffrey P. Sutton, M.D., Ph.D. (<i>Ex Officio</i>) NSBRI Director	W. Dalton Tomlin (Secretary/Treasurer) Baylor College of Medicine	Arnold N. Weinberg, M.D. (Emeritus) Massachusetts Institute of Technology
Torsten N. Wiesel, M.D. (Emeritus) Rockefeller University		

7.3 External Advisory Council

The current NSBRI External Advisory Council members appear in Table 4. In FY 2004, the Council met twice in Houston and once in San Diego.

Six new members were added, either filling a vacancy or replacing a member who stepped down. New members included Thomas M. Best, M.D., Ph.D., University of Wisconsin Medical School; Tammy M. Bray, Ph.D., Oregon State University; Kathleen E. Cullen, Ph.D., McGill University; Gloria Rakita Leon, Ph.D., University of Minnesota; Daniel R. Masys, M.D., University of California, San Diego; and Diane L. Schneider, M.D., a consultant to University of California, San Diego.

Three members left the EAC. M. Rhea Seddon, M.D., Vanderbilt University, rotated off the Council having served five years. Muriel D. Ross, Ph.D., University of New Mexico Health Sciences Center, stepped down due to other obligations, and Dennis S. Charney, M.D., stepped down after joining the faculty of Mount Sinai School of Medicine (consortium members cannot be part of the EAC).

7.4 Board of Scientific Counselors

NSBRI management worked with the Board of Scientific Counselors and NASA Peer Review Services to complete an electronic process for submitting annual progress reports to NSBRI. The reports emphasize productivity, schedule and cost to meet the specific aims of a project, and also stress progress towards a higher CRL/TRL in addressing enabling questions on the Bioastronautics Roadmap. The reports satisfy NASA's requirements for the Task Book, as well as provide additional information on deliverables relevant to exploration.

Work on a system for online secure assessment of project and team progress also took place. The metrics will be presented at the next BSC meeting in October 2004. The BSC was active in peer review of proposals submitted in response to NRA 03-OBPR-04, as well as other open calls. The Chairman of the BSC continues in an *ex officio* role on the EAC.

7.5 User Panel

NSBRI made closer ties to the Astronaut Office through involvement with the Bioastronautics Science Management Team, Space Medicine Liaison and Behavioral Health Liaison. Astronauts also participated in several discipline Research Team meetings. The User Panel's composition is currently being updated to include astronauts who have flown on long-duration missions, especially on the International Space Station.

8.0 Supporting Programs

8.1 Industry Forum

Current members of the Industry Forum are shown in Table 5. During the year, Joseph Kerwin, M.D., retired from Wyle Life Sciences following a long and distinguished career. Dr. Kerwin was the founding director of the NSBRI Industry Forum and has been actively involved in the leadership of the Forum since its inception. David Strome, Ph.D., will represent Wyle Life Sciences Division on the Industry Forum, as NSBRI actively engages in recruitment of new leadership to replace Dr. Kerwin. Mark Wilson will continue as Executive Secretary of the Forum.

Table 4.**NSBRI External Advisory Council**

Lawrence A. Palinkas, Ph.D. (Chairman) Professor Family and Preventive Medicine University of California, San Diego	Leon Alkalai, Ph.D. Director Center for Integrated Space Microsystems Jet Propulsion Laboratory	Ruth Benca, M.D., Ph.D. Professor and Associate Chair Department of Psychiatry University of Wisconsin Medical School
Theodore W. Berger, Ph.D. Professor Department of Biomedical Engineering University of Southern California	Thomas M. Best, M.D., Ph.D., FACSM Associate Professor Orthopedics and Rehabilitation University of Wisconsin Medical School	Tammy M. Bray, Ph.D. Dean and Professor College of Health and Human Sciences Oregon State University
Thomas F. Budinger, M.D., Ph.D. Professor and Chair Department of Bioengineering Lawrence Berkeley National Laboratory	Gilbert A. Castro, Ph.D. Vice President for Inter-institutional Relations and Health Affairs University of Texas Health Science Center at Houston	Kathleen E. Cullen, Ph.D. Associate Professor Department of Physiology McGill University
Stephen Doty, Ph.D. Director, Analytical Microscopy Core Facility Hospital for Special Surgery	Thomas A. Fleisher, M.D. Chief, Department of Laboratory Medicine National Institutes of Health	Eileen M. Hasser, Ph.D. Professor, Dalton Cardiovascular Research Center University of Missouri-Columbia
Gregory T. Kovacs, M.D., Ph.D. Associate Professor, Electrical Engineering Stanford University School of Medicine	Amy Kronenberg, Sc.D. Group Leader Radiation Biology and Environmental Toxicology Lawrence Berkeley National Laboratory	Gloria Rakita Leon, Ph.D. Professor Department of Psychology University of Minnesota
Daniel R. Masys, M.D. Director, Biomedical Informatics Professor of Medicine University of California, San Diego	Donna M. Murasko, Ph.D. Professor and Interim Dean, College of Arts and Sciences Bioscience and Biotechnology Drexel University	Carolyn F. Randolph, Ph.D. Assistant Executive Director South Carolina Education Association
Irwin H. Rosenberg, M.D. Professor, Medicine and Nutrition Dean, Nutrition Science & Policy Tufts University	Diane L. Schneider, M.D. Consultant University of California, San Diego	William Schwartz, M.D. Professor of Neurology University of Massachusetts Medical School
Charles M. Tipton, Ph.D. Professor Emeritus Department of Physiology University of Arizona	F. Eugene Yates, M.D. Professor Emeritus Medicine and Medical Engineering Geriatric Research University of California, Los Angeles	Bobby R. Alford, M.D. (<i>Ex Officio</i>) Chancellor Baylor College of Medicine
Hal E. Broxmeyer, Ph.D. (<i>Ex Officio</i>) Chairman and Mary Margaret Walther Professor Microbiology/Immunology, Medicine Indiana University School of Medicine	Jeffrey P. Sutton, M.D., Ph.D. (<i>Ex Officio</i>) NSBRI Director	

Forum activities for the year included a productive meeting with Novartis, held in Basel, Switzerland, to discuss the usefulness of zoledronic acid as a countermeasure for bone loss during extended space missions. As mentioned previously, this research is the subject of a Supplemental Medical Objective undergoing review. The meeting was attended by John Caminis, M.D., Novartis representative to the NSBRI Industry Forum, Dr. Sutton, Dr. Kerwin and Bone Team member Jay Shapiro, M.D. The trip was successful in increasing Novartis management's commitment and support for in-kind contributions and for future cooperative research efforts between NSBRI and Novartis. A Forum teleconference occurred in March.

The Industry Forum was represented on the NSBRI Postdoctoral Fellowship Review Committee, with Mark Wilson's participation as a panel reviewer. In the coming year, there will be increased efforts to facilitate planned Industry Forum co-sponsorship of NSBRI Postdoctoral Fellowships.

Table 5. NSBRI Industry Forum

The Boeing Company
The Charles Stark Draper Laboratory
Lockheed Martin Astronautics
MBI International
Novartis Pharmaceuticals Corporation
Payload Systems, Inc.
Raytheon Technical Services Company
Roche Laboratories, Inc.
SGI
Southwestern Bell
United Space Alliance
Veridian
Wyle Laboratories

8.2 Bioinformatics

As outlined in the Revised Strategic Plan, the Institute supports the development and implementation of a distributed, but integrated, data management system for ground research, medical and space flight studies. In support of this initiative, significant efforts have been devoted toward implementing metrics for the NSBRI Science and Technology Pipeline, which details advancements in Institute-sponsored projects for countermeasure research and development, testing, evaluation and operational integration. Coordinated tracking of countermeasure and technology development for each project is done according to investment per product line in areas of Human Health Countermeasures, Autonomous Medical Care, Behavioral Health and Performance, and Radiation Health. Additional assignment of Institute deliverables is incorporated into the pipeline matrix according to critical risk levels, technology risk levels, as well as Bioastronautics Roadmap and design reference mission mapping, and alignment with exploration spiral development strategy.

This coordinated approach is being applied to critical risks to provide a substrate for future refinements in multiple areas for health care maintenance and support during exploration-class missions. Moreover, these data are applicable towards enhancing integrative research within and

across the research teams. The addition of Daniel Masys, M.D., Director of Medical Informatics at the University of California, San Diego, to the NSBRI EAC will enhance Institute efforts in support of bioinformatics.

8.3 Communications and Outreach

In support of NSBRI's mission, the Communications and Outreach Office develops and implements diverse communications and outreach initiatives. The program identifies and targets messages to the NSBRI's key publics – the scientific community, industry, consortium members, the public and NASA. Key activities in FY 2004 included the implementation of a quarterly electronic newsletter, *NSBRI Explorer*, focused pitches to news media on specific areas of NSBRI expertise or specific programs, collaboration with public affairs offices at all funded institutions to maximize news outreach related to NSBRI's newly selected research projects, continued collaboration with JSC public affairs to increase awareness of NSBRI activities, and a presentation by Dr. Jeanne Becker at NASA's "Fit for Space" Media Workshop.

On the eve of President Bush's announcement of the new space initiative, Communications and Outreach proactively issued a media advisory to high profile newspapers and magazines. The pitch to media resulted in requests for NSBRI expert interviews by the Associated Press, Baltimore Sun, New York Newsday, New York Times, San Jose Mercury News, Denver Post, Boston Globe, Oakland Tribune, San Francisco Chronicle, Philadelphia Inquirer, Hartford Courant, Tampa Tribune, Orange County Register, Ottawa Citizen, Smithsonian Magazine, and the Association of American Medical College's *AAMC Reporter*. At least 36 documented news stories resulted.

In continued efforts to target publications reaching a science literate audience that might not be well informed about space research, key placements on NSBRI research included Scientific American, The Lancet Neurology, Wired News, and Monitor on Psychology. A targeted effort to increase visibility for NSBRI's education efforts resulted in nine feature articles on the Institute's Summer Internship Program participants.

In FY 2004, the Institute received 204 media inquiries seeking interviews with NSBRI investigators, up from 107 the previous year. NSBRI documented 115 newspaper, magazine or on-line articles mentioning the Institute's work or citing its experts. Since the NSBRI does not utilize a news clipping service, the clipping figure is based on material received from reporters and other sources. Subscriptions to the NSBRI's Web-based E-News Service increased to 912, up from 653 at the end of FY 2003.

9.0 Institute Diversity and Scientific Community Outreach

The Institute remains committed to its leadership role in ensuring excellence in its programs, with strong positions in supporting diversity and outreach. Outstanding scientific, technical and clinical leaders who are woman and/or minority representatives have been appointed to key Institute positions. For FY 2004, these positions include the Associate Director and Chief Scientist, Behavioral Health Liaison, Team Leadership, members of the Board of Directors, External Advisory Council, Board of Scientific Counselors and specialist positions within NSBRI Headquarters.

The research and education requests for proposals were open, national solicitations. Similarly, the call for team leadership positions was an open, national solicitation. The peer review panels had excellent geographical, ethnic and gender diversity. Fifty percent of the Postdoctoral Fellows

selected for funding were women, and the Summer Interns represented a diverse group of potential future space life scientists.

Scientific community outreach is integrated throughout NSBRI activities. The Education and Public Outreach Team and the NSBRI Headquarters based Communications and Outreach Group continue to broadly disseminate information on NSBRI research and the space life sciences. The Institute's Web site averaged 108,000 page requests per month, and the Institute outreach remains well coordinated and effective with the academic institutions and NASA in reaching a large national audience.

10.0 Special Projects

The Cooperative Agreement Management Plan (CAMP) between NASA and the NSBRI enables the partners to undertake special projects outside the core funding of NSBRI. During FY 2004, two new projects began.

The Visiting Scientist/Research Associate Program is an ongoing program that provides young and established university-based researchers with an opportunity to work side-by-side with government employees in JSC laboratories. In addition, there are several ongoing projects including an Operational Hearing Conservation Project, a Medical Informatics and Health Care Systems Project, a Habitability and Environmental Factors Project, and a Space Radiation Health Project Office project. Table 6 provides a list of the participants in these programs as of September 30.

Table 6. Visiting Scientist/Research Associate Program – FY 2004

Name	NASA Program Area	Period
Johnny Conkin, Ph.D.	Environmental Physiology Laboratory	6/1/98 – current
Richard Danielson, Ph.D.	Operational Hearing Conservation Project	1/13/03 – current
Dominick D'Aunno, M.D. (part time)	Cardiovascular Physiology Laboratory	11/1/97 – current
Meena Husein	Medical Informatics and Health Care Systems Office of the Medical Sciences Division	6/1/01 – current
Ralph Krog, J.D.	Medical Informatics and Health Care Systems Office of the Medical Sciences Division	9/17/01 – current
Lawrence H. Kuznetz, Ph.D.	Human Adaptation and Countermeasures Office	8/20/01 – current
Ajitkumar Mulavara, Ph.D.	Neuroscience Laboratory	8/20/01 – current
Michele Perchonok, Ph.D.	Habitability and Environmental Factors Office	9/5/00 – current
Sudhakar Rajulu, Ph.D.	Habitability and Environmental Factors Office	4/17/00 – current
M. G. Sriram, Ph.D. (<i>Moved to University of Texas</i>)	Medical Informatics and Health Care Systems Office of the Medical Sciences Division	12/9/02 – current
Cary J. Zeitlin, Ph.D.	Space Radiation Health Project Office	5/13/02 – current

Selected highlights of Visiting Scientist/Research Associate Program:

- Dr. Sutton and NSBRI/NASA Space Medicine Liaison, Dr. Clark, met with members of the JSC Medical Informatics group, who are part of the NSBRI/NASA translational workforce. The discussion focused on the need for a handbook that would link current space medicine capabilities with known Bioastronautics research, including research in the NSBRI pipeline.

- Richard Danielson, Ph.D., was invited to serve on an Institute of Medicine and National Research Council committee – The Committee on Assessment of Acoustic Trauma Associated with Military Service.
- Michele Perchonok, Ph.D., worked with the NASA Destination Tomorrow crew to develop a script on the challenges of developing foods and packaging for long-duration missions of the future. The episode was taped during the week of August 16.

During FY 2004, two special projects joined the NSBRI portfolio.

Project 04-01 – *Tactical Planning and Integration*, is a project designed to address three specific goals.

- Develop a tactical planning and integration team capability with senior leadership of NASA and NSBRI that will provide an interface, and leadership as required, for NASA JSC/NSBRI to fully implement Bioastronautics tactical planning and implementation.
- Conduct a fully joint effort to ensure overall integration of multiple inter-NSBRI/NASA, intra-NASA JSC, and inter-NASA/Center efforts. The last integration effort will be dependent upon the final roles and responsibilities assigned by NASA HQ to NASA JSC.
- Provide processes that identify and accelerate the transfer of NSBRI-developed knowledge and technologies against the Bioastronautics Roadmap.

Project 04-02 – *Math Missions: Moon to Mars and Beyond*, is an education project to begin the process of showing how an understanding of the cross disciplinary use of mathematics:

- Is central to both understanding and conducting science;
- Can be used to bolster the biosciences and serve to demonstrate their important role in biological research; and
- Can provide grade-specific, hands-on, inquiry-based, and education-standard driven (non-abstract) working models of the use of mathematics.

This project will expand and enhance the mathematical aspects of science education materials developed by NSBRI's *Defying Gravity: Embracing Life in Space* project. The existing materials have already been prepared and tested. Using the existing materials is advantageous in producing well-rounded and comprehensive learning materials reflective of the NSBRI and NASA basic science, research and inherent mathematics.

11.0 Future Directions

NSBRI is successfully implementing its Revised Strategic Plan, and countermeasure research and development continues on, or ahead of schedule, and within cost, toward high CRL/TRL deliverables. The program maps well to the strategy being laid out by the Exploration Systems Mission Directorate. Going forward, NSBRI will continue to work with Johnson Space Center, other NASA Centers and NASA Headquarters, to ensure that the investment, value and productivity of NSBRI for NASA, in achieving goals for human exploration, are realized.

The Institute will expand ground-based validation in a variety of analog models, including bed-rest and KC135 testing, in the CRL/TRL range from 3 to 7. NSBRI will continue to support the development of Supplemental Medical Objectives and other Countermeasure Evaluation and Validation Project approved studies for flight that are generated from NSBRI-sponsored research. NSBRI will continue its team and product integration efforts with NASA and industry, through a new facility (the NSBRI Integration Facility), which is in close geographical proximity to Johnson Space Center. Efforts will continue to link NSBRI research and development to evolving program requirements within the Exploration Systems Mission Directorate, and NSBRI will continue its

leadership in strategic roadmap activities and in tactical implementation of a medical technology maturation program.

Further refinement of the Education and Public Outreach Program will be pursued, with the continued expansion of the training component via development of a Graduate Education Program leading to a Ph.D. in Space Life Sciences, and by support of Postdoctoral Fellows on an annual basis. A series of metrics for evaluation of educational progress will be pursued, in accordance with the National Science Education Standards, to ensure the highest level of quality in production of educational products. Increased integration of educational program efforts with the research team activities will also be encouraged and supported. Broad leadership for the educational program as a whole will be sought during the coming year, in order to coordinate these efforts across all levels of training, with the overarching goal of creating a continuous pipeline from kindergarten to independent investigator.

Appendix A

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

Relevance Assessment Scale

I. Strategic Need

- *High:* Proposed work is highly relevant to the NRA and the NSBRI Team Strategic Plan. There is clear added value of the proposed project to the NSBRI Research Program.
- *Medium:* Proposed work is relevant to either the NRA or the NSBRI Team Strategic Plan, but is not highly relevant to both. There is some added value of the proposed project to the NSBRI Research Program.
- *Low:* Proposed work is weakly relevant to either the NRA or the NSBRI Team Strategic Plan. There is no added value of the proposed project to the NSBRI Research Program.

II. Product Development

- *Excellent:* A superior plan and high likelihood for progress along CRL/TRL scales toward useful deliverables (e.g. knowledge, risk assessors, operationally valid countermeasures, enhanced medical capabilities)
- *Satisfactory:* A plan for moderate progress along CRL/TRL scales toward deliverables.

III. Return on Investment

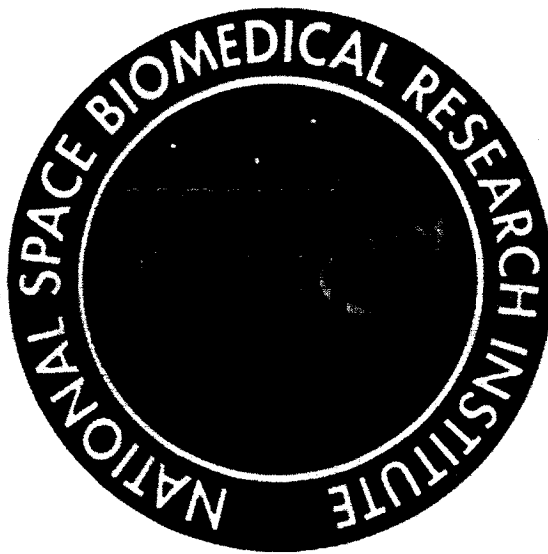
- *Superior:* Good to excellent value on dollar investment toward answering critical questions and achieving Institute's goals and objectives.
- *Moderate:* Fair to average value on dollar investment toward answering critical questions and achieving Institute's goals and objectives.

Scientific Merit

Version 1.0

Appendix B

**NATIONAL
SPACE BIOMEDICAL
RESEARCH INSTITUTE**



***CORE RESEARCH PROGRAM
YEAR 7 - FY 2004***

September 30, 2004

**National Space Biomedical Research Institute
Core Research Program – Year 7
FY 2004
September 30, 2004**

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NSBRI RESEARCH PROGRAM BONE LOSS

Team Leader:	Cavanagh, P. R.		The Cleveland Clinic Foundation	
Associate Team Leader:	Bloomfield, S. A.		Texas A&M	
Bateman, T. A.	PI	Clemson	Examination of Anti-Resorptive and Anabolic Treatments/Stimuli on Unloading-Induced Osteoporosis	5
Norrdin, R. W.	CO-I	Colorado State		
Simske, S. J.	CO-I	Univ. of Colorado		
Bloomfield, S. A.	PI	Texas A&M	Bone and Muscle Recovery from Simulated Microgravity (End Date: 02/29/04)	6
Hogan, H. A.	CO-I	Texas A&M		
Smith, C. L.	CO-I	Baylor		
Bloomfield, S. A.	PI	Texas A&M	Increasing the Efficiency of Exercise Countermeasures for Bone Loss	8
Baldwin, K. M.	CO-I	UC, Irvine		
Hogan, H. A.	CO-I	Texas A&M		
Bolander, M. E.	PI	Mayo Clinic	Effect of Microgravity on Fracture Healing: Ultrasound as a Possible Countermeasure (End Date: 05/31/04)	9
Turner, R. T.	CO-I	Mayo Clinic		
Greenleaf, J. F.	CO-I	Mayo Clinic		
Cavanagh, P. R.	PI	Cleveland Clinic	Foot Reaction Forces During Simulated ISS Exercise Countermeasures	10
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Isales, C. M.	PI	MCG Research	Therapeutic Modulation of Systemic Glucose-Dependent Insulinotropic Peptide Levels to Counteract Microgravity-Induced Bone Loss	11
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Majeska, R. J.	CO-I	Mount Sinai		
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Toerge, J. D.	CO-I	Nat. Rehab. Hosp.		
Baldwin, K. M.	CO-I	UC, Irvine		
Ruff, C. B.	CO-I	Hopkins/SOM		
Beck, T. J.	CO-I	Hopkins/SOM		
Oden, Z. M.	CO-I	UT-Houston		
Potember, R. S.	CO-I	Hopkins/APL		
Burman, K. D.	CO-I	Wash. Hosp. Ctr.		
Ballard, P. H.	CO-I	Nat. Rehab. Hosp.		
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RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Ted A. Bateman, Ph.D.
ORGANIZATION:	Clemson University
PROJECT TITLE:	Examination of Anti-Resorptive and Anabolic Treatments/Stimuli on Unloading-Induced Osteoporosis

Project Executive Summary

Studies examining the calcium balance during extended space flight on the Russian Space Station Mir suggest that 1) both bone resorption and formation are altered to adversely affect bone density, and 2) it may take bone mass considerably longer to return to preflight levels than the period of microgravity exposure. Countermeasures to prevent the loss of bone mass and therapies to more quickly replace bone postflight are desirable to 1) not only prevent fractures and kidney stones during and shortly after long-duration missions, but 2) to also prevent the microgravity-associated bone loss from exacerbating natural osteoporosis as an astronaut ages. However, despite the severity of bone loss and the long-term health concerns, with so many biomedical problems caused by space flight, every effort should be made to develop countermeasures that require minimal intervention.

This proposal outlines the development of a treatment regimen that both prevents inflight bone loss and accelerates the recovery of bone health postflight. Both bone mass and turnover rates are important for evaluating successful recovery. This will be achieved by first demonstrating that low doses of anti-resorptive agents can both prevent the inflight loss of mass, but also by allowing turnover to recover more quickly. This will be examined in mice for two anti-resorptive agents: osteoprotegerin (OPG) and zoledronate.

For the second phase of the project, OPG will be combined with anabolic agents. Two disuse osteoporosis models, followed by a period of loaded recovery, will be used. Low-dose OPG will be administered during unloading and the anabolic therapeutic will be examined during loaded recovery. The anabolic agents being examined are also designed to develop a minimally invasive regimen: exercise, ultrasound and parathyroid hormone (PTH), in this order, are the preferred methods of returning bone health. Exercise is already an important component to postflight reconditioning in astronauts. Its contribution to returning bone health will be characterized. Ultrasound is a clinically approved therapy for aiding fracture repair. It may be able to return local, at-risk areas of the skeleton (i.e. vertebra), to preflight levels. Finally, PTH is the only clinically approved anabolic therapy for osteoporosis and may be necessary in cases where an astronaut has lost an unusually large amount of bone or when turnover is not returning to preflight levels.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Susan A. Bloomfield, Ph.D.
ORGANIZATION:	Texas A&M University
PROJECT:	Bone and Muscle Recovery from Simulated Microgravity
END DATE:	2/29/2004

Project Executive Summary

These investigations are designed to determine how mechanical interactions between bone and skeletal muscle contribute to the recovery of bone mass and bone strength after exposure to prolonged microgravity. Given evidence for the risk of bony injury when large muscle contractile forces are imposed on weakened bone, these are key issues to solve to minimize risk of injury to human astronauts during and following exploration class missions. The consequences of this disrupted muscle-bone balance for the recovery of bone after skeletal unloading are not well understood. Hence our first objective was to define a time course for recovery of both skeletal muscle and bone functional properties after 28 days of hindlimb unloading (HU) in an adult rat model. The data clearly indicate that bone mineral density (BMD) at the proximal tibia, a skeletal site sensitive to changes in mechanical loading, decreases about 5 percent over 28 days of HU, and declines even further during the next 28 days of normal ambulation (to about 92 percent of baseline values). On the other hand, strength of the ankle plantar-flexor muscles declines more rapidly during HU but then by 14 days of recovery has recovered to 98 percent of baseline values. Force-frequency relationships are also fully normalized by 14 days of recovery. Hence there is a clear functional mismatch between these two tissues between 14 and 28 days of recovery, with BMD (a surrogate measure of bone strength) still declining and muscle function recovered to 110 percent of baseline values by 28 days of cage activity. Other outcome measures should help define tissue level mechanisms promoting or retarding recovery of bone following unloading, including the role of bone marrow osteoprogenitor cells. Thus far, our cell culture data do not present a clear picture of the functionality of these precursor cells; more work remains to be done here. Histomorphometry studies of mid-tibia cortical bone reveal that bone formation rate at the periosteal surface is nearly shut down after 28 days of HU, but recovers nicely after 28 days of normal ambulation. Similar studies of proximal tibia cancellous bone now in progress should reveal in year three if there is any recovery of normal bone formation activity in this bone compartment that usually responds more rapidly to changes in loading.

We have begun studies on the effectiveness of several mechanical loading regimens and two pharmacological agents in promoting bone recovery and minimizing the functional mismatch between bone and muscle after 14 days of recovery. Preliminary results indicate that parathyroid hormone (PTH, a formulation of which is now FDA-approved for clinical use in humans) is remarkably effective in promoting recovery of proximal tibia BMD during recovery from 28 days of HU. Important groundwork is described in this progress report towards testing a mechanical loading intervention during recovery which utilizes stimulated muscle contractions, in an attempt to answer whether intensive weight training (in a human returned from spaceflight) can safely increase BMD while also hastening muscle recovery.

Our collaborative group has published two articles in the past year resulting directly from this funding, with another manuscript in preparation. Research presentations on these data were made at the American Society for Bone and Mineral Research (9/03), the Bioastronautics meetings (1/03) and Experimental Biology meetings (4/03).

Interim results and budget cuts during year two delayed progress in testing some of the interventions and will likely result in dropping the aerobic exercise intervention during recovery during year three. However, since most available data on rodents and humans indicate that resistance-type training is usually superior for minimizing bone loss with disuse, we feel this is a minor disadvantage.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Susan A. Bloomfield, Ph.D.
ORGANIZATION:	Texas A&M University
PROJECT TITLE:	Increasing the Efficiency of Exercise Countermeasures for Bone Loss

Project Executive Summary

The goal of the research is to test the hypothesis that astronaut exercise time designed to mitigate bone loss can be reduced if combined with the appropriate pharmacological agent. The specific objectives are to define an optimal exercise regimen that will minimize bone loss during prolonged unloading and then to test reduced doses of that exercise regimen in combination with alendronate administration. Importantly, skeletal muscle function, mass, and phenotype will be simultaneously tested to assure that any reductions in exercise time do not sacrifice benefits to muscle. These experiments utilize the well-accepted model of hindlimb unloading (HU) in mature adult rats in order to test these concepts in a far more comprehensive way than is possible with human subjects.

Specific aim one will test two different modes of loading (electrically stimulated muscle contractions and voluntary resistance exercise) for their effects on mitigating bone and muscle decrements during 28 days of HU. Outcome measures include in vivo tests of bone density/geometry and muscle function, and detailed ex vivo studies of bone mechanical properties, histomorphometric variables, and skeletal muscle anabolic/catabolic state.

The regimen judged most successful in mitigating bone loss will be further refined in specific aim two, by testing varying frequency or session durations during HU. In addition, a dose-response study using alendronate during HU will be performed. That volume of training producing the maximal benefit to bone and acceptable benefit to muscle will then be tested in combination with the minimal dose of alendronate producing significant attenuation of bone loss.

Specific aim three will test systematically-reduced volumes of the optimized loading regimen in combination with alendronate to determine what reduction in exercise time can be achieved without sacrificing benefits to bone and muscle. These integrated experiments address six critical questions on the Critical Path Roadmap relevant to bone health and skeletal muscle function. They will also provide fundamental and unique information about the biological interaction of these two tightly integrated tissues in response to the anabolic stimulus of exercise and the anti-resorptive effects of alendronate. The outcomes of these animal protocols can be easily translated to human studies using appropriate ground- and flight-based test beds.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Mark E. Bolander, M.D.
ORGANIZATION:	Mayo Clinic Rochester - Rochester
PROJECT TITLE:	Effect of Microgravity on Fracture Healing: Ultrasound as a Possible Countermeasure
END DATE:	5/31/2004

Project Executive Summary

The NSBRI Conference convened in Clear Lake, Texas, identified fracture healing during space flight as an area where further information would be required to appropriately prepare for long-term space missions, and developing countermeasures to restore normal fracture healing was identified as a priority for current research.

Our current understanding of bone physiology suggests that fracture healing will be abnormal in the microgravity environment. This hypothesis is supported by two published studies, the first an abstract reporting abnormal healing in rats undergoing hindlimb unloading, the second a manuscript (in Russian) that describes abnormal fracture healing in five rats with fibula fractures flown on Cosmos-2044. This latter study reports that abnormalities seen in fracture healing after space flight were duplicated in the hindlimb-unloading model.

The goals of the experiments are 1) to confirm the previous reports that microgravity adversely affects fracture healing, and 2) to determine if ultrasound treatment, which has been shown to accelerate fracture healing in clinical studies, will reverse the impaired cellular events in fracture healing that are related to microgravity. If ultrasound does not act as an effective countermeasure we will undertake detailed evaluation of our histologic samples to identify potential targets for other countermeasures.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Peter R. Cavanagh, Ph.D., D.Sc. (Med)
ORGANIZATION:	The Cleveland Clinic
PROJECT TITLE:	Foot Reaction Forces During Simulated ISS Exercise Countermeasures

Project Executive Summary

Three exercise countermeasure devices are now on the ISS (TVIS, CEVIS, iRED), and thus they are assumed to be at countermeasure readiness levels eight or nine. However, it is now known that astronauts on the first six expeditions to the ISS lost lower-extremity bone mass despite exercising on these devices. Furthermore, there is no information in the literature to demonstrate the frequency content and magnitude of the loads (F) and rate of change of loads (dF/dt) imposed on the lower extremities during exercise throughout the range of available settings. F and dF/dt are widely believed to be important criteria for efficacy of countermeasures against loss of bone mass.

As the countermeasure devices are used and upgraded during their anticipated 10-year life span, there is a need to establish their loading characteristics in a realistic ground-based simulation so that exercise prescriptions and the design of components such as the harness and subject load device (SLD) can be optimized. In particular, the compliant nature of the interface between the device and its attachment to the ISS has an important influence on F and dF/dt and has not yet been adequately included in previous simulations. To address these issues, we have assembled a powerful multidisciplinary team of investigators with backgrounds in simulation, space engineering, human biomechanics, in-flight experimentation, astronaut conditioning and skeletal mechanics. The unique facilities of the Cleveland Clinic's Biomechanics Laboratory and NASA Glenn Research Center's Microgravity Emissions Laboratory will be available to the proposed project.

Following investigation of how the interface parameters affect biomechanical loading, we propose to enhance the Zero Gravity Locomotion Simulator (ZLS) at the Cleveland Clinic to provide a more flight-like simulation of exercise. We will then define the entire range of loading possibilities in cycling, treadmill running, and resistance exercise using existing and improved SLDs and the improved ZLS, which will be flexible enough to accept new countermeasure devices as they emerge. We will use the results from the experiments in the design of countermeasures using theories of the relationship of bone mineral density to daily loading. The results of this project will provide clear guidelines for the prescription of in-flight exercise on existing devices that can be projected to be protective against bone and muscle loss based on a directly measured mechanical "dose."

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Carlos M. Isles, M.D.
ORGANIZATION:	Medical College of Georgia
PROJECT TITLE:	Therapeutic Modulation of Systemic Glucose-Dependent Insulinotropic Peptide Levels to Counteract Microgravity-Induced Bone Loss

Project Executive Summary

Our long-term goal is to understand the molecular mechanisms of bone formation, maintenance and repair. Weight-bearing is essential for bone formation and maintenance. In the absence of load, for example, in the microgravity environment of space, bone tends to atrophy. We hypothesize that the detrimental effect of diminished gravitational load can be overcome by exploiting the hormonal cues received by bone. Our primary candidate for effecting this control is the enteric hormone, glucose-dependent insulinotropic peptide (GIP), secreted from the small intestine in direct response to nutrient intake. The accepted function of GIP is coupling insulin secretion to blood glucose elevations. However, our group was the first to demonstrate an additional function for GIP, namely the stimulation of bone formation(1-3). Functional GIP receptors are present on bone-forming cells *in vivo* and activation of these GIP receptors leads to an increase in bone formation and, conversely, an inhibition of bone resorption.

As part of our NSBRI funded project we have performed experiments exploring different levels of hormonal control of bone formation as potential sites of GIP action. We have found important new controls of bone formation both produced systemically (endocrine control) and locally (paracrine control). Findings to report for this annual report include: (1) new findings on the molecular controls of bone formation (2) new findings on how nutrients we ingest can be directed to the bones to be utilized for bone formation and (3) the discovery of a novel hormonal loop in bone where the bone resorbing cells, osteoclasts, regulate the bone forming cells, osteoblasts, through local production of bone active hormones.

(1) Molecular controls of bone formation: Our work in this area has focused on two factors *Tbx2* and ERV-9 LTR. *Tbx2* belongs to a family of developmental transcription regulatory factors. We evaluated whether the gap junction protein Connexin43 (Cx43), an important regulator of osteoblast function and bone development, may be a downstream target gene regulated by *Tbx2*. Our findings indicate that the promoter of Cx43 is repressible by *Tbx2*, both in cultured osteoblast-like cells *in vitro* and also likely in the developing embryo. We have found that parathyroid hormone can modify *Tbx2* expression and are now evaluating whether it is also modified by GIP.

The long terminal repeats (LTRs) of the human endogenous retrovirus ERV-9 is different from other endogenous retroviruses in that they have recurrent motifs capable of binding to cognate transcription factors. We have found that embryonic bone stem cells (but not mature bone cells) contain these ERV-9 LTRs suggesting a role in bone cell development. We have begun the characterization of bone stem cells and plan to define the role of environmental factors on bone stem cell development.

(2) Nutrient utilization for bone formation: Our work in this area has focused on GIP as an integrative hormone with effects on both the vasculature (to maximize postprandial nutrient absorption) and effects on the bone to stimulate new bone formation. We propose that by therapeutically elevating GIP levels, coupled with strict dietary control, it will be possible to mitigate the impact of microgravity.

It is well known that after a meal the blood flow to our hepatic artery decreases and the portal vein circulation increases, the purpose of this alteration in blood flow is to increase the absorption of nutrients from the recently ingested meal. These changes can be reproduced by infusion of GIP, suggesting that GIP is the hormone responsible for these changes in blood flow after a meal. In order to determine how GIP could have apparently opposing effects on blood flow in these different vascular beds we isolated primary hepatic artery and portal vein endothelial cells and found that GIP's action differed between these endothelial cells. In portal vein, but not hepatic artery endothelial cells, GIP increased production of the potent vasodilator nitric oxide. In contrast, in hepatic artery, but not portal vein endothelial cells, GIP increased release of the potent vasoconstrictor endothelin-1. These findings highlight the importance of GIP as a hormone involved in nutrient utilization.

GIP is also an important hormonal link between nutrient ingestion and bone formation. To define GIP's role in normal bone formation we have generated transgenic mice with GIP levels up to ten times normal. We have examined the bone phenotype in these GIP-overexpressing transgenic mice and find that they have a significantly increased bone mass and the bones of these animals, assessed by biomechanical testing, are stronger than those of control mice. We are currently performing tail suspension experiments in these animals. Our hypothesis predicts that these mice will be protected against bone loss in the simulated microgravity environment.

Experiments to be performed over the coming year should give us a much better understanding of GIP's effects on bone. We have obtained GIP receptor knockout mice from Dr. Yamada in Japan and as predicted these mice have about a 13 percent lower bone mass than normal control mice. We are currently performing tail suspension experiments on these mice; we would predict that these mice, having lost the protective effect of GIP on bone formation, would be much more prone to lose bone under simulated microgravity.

(3) Paracrine control of bone turnover: Our work in this area has focused on two hormones, PACAP and ACTH.

PACAP (Pituitary Adenylate Cyclase Activating Peptide) is known to regulate proliferation, differentiation, and apoptosis in some cell populations. In addition, PACAP regulates metabolism and the cardiovascular, endocrine, and immune systems although, specific PACAP actions on many tissues is still poorly defined. The PACAP receptor belongs to the seven transmembrane G-protein coupled family of receptors that also includes GIP, PTH/PTHrP, calcitonin, secretin, VIP and GLP-1. We have found data specific high affinity PACAP receptors in osteoblastic-like cells and our data suggests that PACAP may play a role in osteoblastic proliferation and differentiation. Thus, our data suggests another mechanism for nutrient regulation of bone formation, through PACAP release from nerves innervating the bone, activated upon nutrient ingestion.

Finally, we have done experiments examining a role for ACTH in bone formation. Another nutrient related hormone which has been reported to have bone specific effects is leptin. Leptin

ultimately acts through the melanocortin receptors. Melanocortin receptors belong to the seven transmembrane domain, G-protein coupled family of receptors. Five melanocortin receptors have been described which are widely expressed in the body, including skin, brain, adrenal glands, adipocytes, gut and other peripheral tissues. These receptors are activated by fragments derived from a larger molecule, pro-opiomelanocortin (POMC) and include: ACTH, α β and γ -MSH, and β endorphin. Traditional roles for these peptide hormones include regulation of cortisol secretion, skin pigmentation and pain perception. Although the pituitary gland is the traditional site for synthesis, secretion and processing of POMC-derived fragments, it has become clear that other tissues can also synthesize and secrete POMC fragments including keratinocytes, melanocytes, endothelial cells and immune cells among others. Among the latter both lymphocytes and macrophages have been reported to express and secrete POMC fragments. Since osteoclasts in bone are derived from macrophages we investigated whether POMC and the melanocortin receptors were expressed in bone and bone cells. We found that the five known melanocortin receptors are expressed in normal rat bone as assessed by *in situ* hybridization. These receptors were variably expressed in different osteoblastic-like cell lines with the MG63 cell line expressing four out of five melanocortin receptors. In attempt to determine the source of ligand for these melanocortin receptors, normal rat osteoclasts were probed for the POMC gene by Northern blot. Osteoclasts did in fact express the POMC gene although at a lower level than normal rat pituitary and conditioned osteoclastic tissue culture medium contained ACTH. Thus, bone cells contain the elements for a paracrine hormonal loop where osteoclasts express POMC and osteoblasts express melanocortin receptors.

Thus, we have made significant new advances in our research objectives this year and have gained new insights into the controls involved in bone formation. During the next calendar year we propose to extend this year's findings and thus, increase the countermeasure readiness level of GIP as a treatment for microgravity-induced bone loss.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Gerard Karsenty, M.D., Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Leptin as a Regulator of Bone Formation in Microgravity
END DATE:	05/31/2004

Project Executive Summary

Original Aims

To determine whether leptin controls bone mass by releasing a humoral substance following its binding to its hypothalamic receptor.

To determine whether the sympathetic nervous system is involved in mediating leptin control of bone formation.

To determine whether a naturally occurring soluble form of the leptin receptor can prevent leptin inhibitory action on bone formation.

Key Findings

Several key findings were made during the previous year of funding. These can be summarized as follows:

Leptin uses different pathways to control bone mass and body weight.

The concentration of leptin in blood is not a good indicator of its action on bone formation.

Neurons present in the hypothalamus and controlling bone formation have been identified.

The mediator coming out of these neurons and affecting bone formation is not present in blood.

Circulating leptin level controls bone mass.

Impact of Findings

These findings confirmed largely our working hypothesis that there is a brain-derived neuronal control of bone mass. They lead us to propose new experiments to identify the mediator relaying information from the brain to the bone cells.

In the coming year we intend to use mutant mouse strains deficient in various neuromediators to identify the mediator of leptin action on bone mass. Once we will have identified this mediator we will generate an inhibitor of this mediation and we will use it in ovariectomized animals to determine whether it can be used to prevent the development of osteoporosis.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Ronald J. Midura, Ph.D.
ORGANIZATION:	The Cleveland Clinic
PROJECT TITLE:	Effects of Simulated Weightlessness on the Repair of Lower-Limb Bone Fractures and on the Number of Bone-Derived Stem Cells

Project Executive Summary

Investigations into the underlying mechanisms of bone loss during extended space flight are high priority objectives of the Critical Path Roadmap of NASA and NSBRI. During prolonged space flight, continuing bone turnover and a reduced capacity to form new bone tissue results in diminished bone mass leading to increased skeletal fragility within select regions of critical bones. Progressive structural compromise in these bone regions will eventually develop fragility fractures even under routine mechanical loads. Furthermore, in the absence of means to increase new bone formation in a space environment, bone fractures will not heal properly placing astronauts at a compounded risk due to reduced physical capacity and secondary medical complications.

The proposed study will employ modern methods of bone biology and imaging to examine bone formation and fracture healing in vivo during simulated weightlessness. Further, it will assess the efficacy of two FDA-approved osteoporosis treatments, parathyroid hormone (PTH) and bisphosphonate therapies, as practical countermeasures to prevent bone loss and promote fracture healing during simulated weightlessness. We will explore cellular mechanisms that may explain why bone formation is reduced and fractures do not heal well in a weightlessness condition.

Specifically, we hypothesize that the detrimental effects of the weightlessness state results in changes in osteogenic progenitor cell growth and differentiation kinetics, and these changes will be manifest in the progenitor cell populations in bone marrow and periosteum at the healing fracture site. Accordingly, we will measure the osteoprogenitor cell numbers in both bone marrow and periosteum, and determine whether a simulated weightlessness condition is associated with a decrease in the number or function of osteoprogenitor cells in these two essential osteogenic tissues. We will also test whether PTH or bisphosphonate therapies will reverse the negative effects on osteoprogenitor cell kinetics and function in a weightlessness condition.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Clinton T. Rubin, Ph.D.
ORGANIZATION:	State University of New York – Stony Brook
PROJECT TITLE:	A Biomechanical Countermeasure for Disuse Osteopenia
END DATE:	07/31/2004

Project Executive Summary

Osteoporosis, the progressive loss of bone density that cripples tens of millions on our planet, distinguishes itself as perhaps the greatest physiologic obstacle to an extended human presence in space. Harnessing bone's strong sensitivity to mechanical signals, there is increasing evidence that extremely low magnitude (<100 microstrain) mechanical signals can be strongly osteogenic if applied at a high frequency (15 to 60 Hz). Such high-frequency, low-magnitude strains comprise a dominant component of a bone's strain history, indicating that these mechanical events represent a significant determinant of bone morphology.

With this in mind, we have been examining if small perturbations in high frequency loading, induced non-invasively into the lower appendicular skeleton, will stimulate an increase in bone mass without sacrificing bone quality. The principal objectives of our research have been to establish the efficacy of this unique, biomechanical countermeasure to inhibit bone loss in an animal model of disuse osteoporosis, and correlate this regulatory influence to the expression patterns of several genes critical to bone formation and resorption. Ten minutes per day of these low-level signals (0.25g), induced non-invasively using an oscillating platform, are able to retain bone mass despite 23 hours and 50 minutes of disuse, while ten minutes of normal weight bearing fails to do to. Longer term animal studies (one year), have shown that low-level mechanical loading, inducing cortical strains on the order of 5 microstrain, can increase cancellous bone volume fraction, thicken trabeculae, increase trabecular number and enhance bone stiffness and strength. Considering these strain levels are far below (<1/1000th) those which may cause damage to the tissue, we believe these signals hold great potential as a mechanical prophylaxis for osteoporosis.

Earth-Based Applications of Research Project

Early clinical trials with the LMMS device, including cerebral palsy children, adolescent girls with osteopenia, or post-menopausal women have been encouraging in their ability to inhibit and/or reverse osteoporosis.

As we move towards further clinical evaluation of this device for the aging and infirm population, as well as consider it for use to curb bone loss in astronauts during long-term space flight, it is clear that this unique intervention affords the ability to examine the molecular basis of an anabolic signal, as well as establish the extent to which non-invasive mechanical signals can provide an effective countermeasure for disuse osteopenia. Importantly, correlating early gene expression to a longer-term bone response will also permit extrapolation of results from short-term space flights to long-term missions.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Mitchell B. Schaffler, Ph.D.
ORGANIZATION:	Mount Sinai School of Medicine
PROJECT TITLE:	Resorption Suppression and Bone Health in Disuse
END DATE:	05/31/2004

Project Executive Summary

Osteoporosis in higher mammals due to loss of normal mechanical loading results from elevated osteoclastic resorption. Thus, targeting osteoclasts to prevent bone loss seems an obvious countermeasure strategy. To that end, during the last two years our NSBRI research (*Resorption Suppression and Bone Health in Long-term Disuse*), we examined whether a clinical bisphosphonate prevented bone loss in dogs subjected to long duration single limb immobilization. We are testing the hypotheses that 1) Long-term suppression of bone remodeling in disuse will successfully maintain bone mass, microarchitecture, stiffness and strength, but will result in compromised fracture resistance properties; and 2) Decreased mechanical usage in the presence of an antiresorptive agent results in loss of osteocyte integrity and accumulation of bone with impaired viability. Bone health is assessed from conservation of bone mass, microarchitecture, tissue mechanical properties and from in situ assessments of osteocyte viability.

To date we have completed the in vivo studies, as well as bone density and histomorphometric analyses. Our studies reveal some important and surprising results. In particular, we found that bisphosphonates were only partially effective in attenuating long-term bone loss resulting from long-term disuse. Risedronate treatment of dogs subjected to long duration single limb immobilization resulted in a 30 to 50 percent reduction in bone loss compared with non-treated disuse animals. While we must consider any conservation of bone in disuse osteoporosis to be beneficial, we do not yet know whether this treatment reduces bone resorption enough such that bone that remains after long-term disuse can make a complete recovery when loading is restored. Moreover, these observations stand in contrast findings for other osteoporoses where bisphosphonates more effectively inhibited bone loss. Thus, these results suggest that disuse is different from other osteoporoses in its sensitivity to anti-resorptive treatment.

In the coming year, we will complete our histomorphometric analyses of tissues. We will also undertake confocal microscopic studies of osteocyte integrity and perform the biomechanical testing needed to determine whether treated bones maintain their mechanical properties in proportion to the amount of bone conserved, and determine whether long-term bisphosphonate treatment adversely affect bone tissue mechanical properties during disuse.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Mitchell B. Schaffler, Ph.D.
ORGANIZATION:	Mount Sinai School of Medicine
PROJECT TITLE:	Bone Recovery Potential After Bisphosphonate and PTH Treatment of Disuse Osteoporosis

Project Executive Summary

Bone loss in microgravity and the resulting bone fragility have been identified by NASA as key barriers to successful long-term space flight. Effective countermeasures must therefore prevent bone loss, but also maintain the mechanical integrity of the tissue during prolonged space flight and allow rapid recovery of normal function.

Disuse osteoporosis in humans and higher mammals results from elevated bone resorption. Thus, targeting osteoclasts with antiresorptive agents, like bisphosphonate to prevent bone loss, is a key strategy. While anti-resorptive drugs have been the cornerstones of osteoporosis therapy, anabolic agents, such as PTH, that stimulate bone formation represent an important new advance in the treatment of osteoporosis. We hypothesize that PTH may be especially valuable in reversing disuse if the deterioration of bone architecture can be slowed such that the anabolic agent has a better initial bone scaffold on which to work.

The studies examine whether bone that remains after bisphosphonate treatment during long-term immobilization can recover its architecture and mechanical function after restoration of mechanical usage (remobilization). We will then assess whether addition of anabolic PTH during immobilization will improve recovery of disuse bone. Recovery after long-term disuse with bisphosphonate treatment will be examined in an immobilization model. MicroCT imaging will be used to evaluate microstructure, biomechanical testing to assess function and histomorphometry to measure tissue physiological responses.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Jay R. Shapiro, M.D.
ORGANIZATION:	Medstar Research Institute
PROJECT TITLE:	Defining and Preventing Bone Loss: A Microgravity Model

Project Executive Summary

Muscle atrophy and bone loss are major complications of spinal cord injury (SCI), chronic bed rest and exposure to microgravity. Space medicine research has amply documented the extent to which muscle and bone loss may impair strength and increase fracture risk. In our research, we theorize that the SCI patient can serve as a surrogate for studying microgravity exposure.

A primary objective of this research program is to limit the extent of bone loss in SCI patients by treatment with a potent intravenous bisphosphonate, zoledronate for a period of one year. The zoledronate effects on bone will be measured using bone density values and femur scan structural analysis as the indicators of bone integrity. We will determine the effects of zoledronate on biomarkers of bone resorption and formation, and on serum calcitropic hormone levels.

To study the process of muscle atrophy when weightless, we will determine the relationships between changes in thigh muscle cross-sectional area measured by CT scan, muscle biopsy immunohistochemistry, muscle protein translation markers and markers for protein synthesis activation and protein degradation. To further understand mechanisms involved in bone loss, we will determine sequential changes in femur bone geometry and structural parameters obtained from DEXA scans by established 2-D curved beam analysis methods. Using femur CT images, we will measure changes in femur bone dimensions and will apply 3-D finite element analysis to estimate fracture risk.

The new time-of-flight mass spectrometer will permit measuring the excretion of zoledronate in urine and plasma levels. We will compare these to radiologic measurements and bone biomarkers. The objectives of this research are:

1. To develop a regimen for minimizing bone loss in SCI subjects that may be appropriate for astronauts during extended microgravity exposure,
2. To investigate mechanisms related to muscle and bone loss during weightlessness, and
3. To explore the SCI patient as a surrogate for the investigation of microgravity induced musculoskeletal atrophy.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Carolyn L. Smith, Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Receptor Countermeasures to Bone Loss in Microgravity
END DATE:	05/31/2004

Project Executive Summary

The prevention of bone loss due to skeletal unloading is a complex problem whose reasons for this loss have not been fully elucidated. The overall goal of the NSBRI bone team is to develop countermeasures that will not only prevent quantitative loss of bone, but also maintain bone strength. Measures that simply prevent resorption may maintain mass, but may block the necessary remodeling that ensures adequate bone strength. Studies to date suggest that good nutrition and exercise regimens will be insufficient to achieve this goal so pharmacological alternatives must be considered. The biological actions mediated by the estrogen receptor (ER) and vitamin D receptor (VDR) play key roles in the normal control of bone growth and skeletal turnover that are necessary for skeletal health. These receptors act by controlling the differentiation and/or function of osteoblasts and osteoclasts, and other cell types within the bone and bone marrow microenvironment, as well as play a role in calcium absorption (VDR). We hypothesize that the appropriate combination of an agent that will improve calcium absorption and encourage bone formation (VDR agonist) along with an agent that will reduce bone resorption (selective estrogen receptor modulators [SERM]) will achieve the goal of maintaining bone mass and bone strength.

To test this we have initiated studies to:

1. Assess the ability of novel receptor agonists of the ER and VDR, alone or in combination, to modulate osteoblastogenesis, mature osteoblast function and osteoclastogenesis in vitro and in vivo, and
2. Assess the ability of novel receptor agonists of the ER and VDR, alone or in combination, to prevent bone loss in the hindlimb suspension model of skeletal unloading.

The effects of unloading and of the countermeasures are being assessed by:

- a. Measuring changes in bone mineral density, histomorphometry, mechanical strength testing and biochemical markers of bone metabolism;
- b. Determining the effects of these treatments on osteoblastogenesis and osteoclastogenesis and function, and
- c. Characterizing gene expression profiles in bone resulting from skeletal unloading and administration of the countermeasures.

Our results to date indicate that ligands of both the ER and VDR possess the ability to attenuate bone loss in the rat hindlimb suspension model of skeletal unloading. Collectively, these studies will lead to a better understanding of the changes associated with skeletal unloading and will test the utility of VDR agonists and SERMS as countermeasures.

Research Impact on America/Earth Benefits

Our findings have impacts on various aspects of research in the United States. First, we now have data to suggest that the responses to treatment with different ligands for the vitamin D receptor may be gender-dependent. While this has implications for astronauts, it is also useful information relative to health care for the general population, and we thus anticipate that this finding may stimulate research on the basis by which the sex-dependent response is achieved.

Our results on the EB1089 treatment of female rats suggests that it may be possible to achieve anabolic effects with a vitamin D receptor ligand, and if this finding is confirmed through subsequent analyses including histomorphometry, this may stimulate further research into the use of agents such as EB1089 for the treatment of post-menopausal osteoporosis. Finally, our results suggest that SERMs such as raloxifene may be an effective treatment for osteoporosis in human males, and this should stimulate further research into the use of this class of drugs in humans.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Carolyn L. Smith, Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Receptor Countermeasures to Bone Loss in Microgravity

Project Executive Summary

The prevention of bone loss due to skeletal unloading is a complex problem and the reasons for this loss have not been elucidated. The overall goal of the NSBRI bone team is to develop countermeasures that will not only prevent quantitative loss of bone, but also maintain bone strength. Measures that simply prevent resorption may maintain mass but block the necessary remodeling to ensure appropriate bone strength. We hypothesize that the appropriate combination of an agent that will improve calcium absorption (VDR agonist) and an agent that will reduce bone resorption (a bone-selective estrogen receptor agonist, STEAR) will achieve these goals.

We have assembled a team of investigators and unique resources to achieve this goal through the use of techniques ranging from state-of-the-art molecular biology to micro-computed tomography (microCT). Dr. Carolyn Smith has the necessary expertise in estrogen receptor modulators, Dr. Nancy Weigel in VDR agonists, Dr. Larry Suva in microCT, Dr. Leif Peterson in microarray analyses and Dr. Zong-Ping Luo in biomechanical testing. Dr. Smith brings additional strength and resources in studying endocrine changes in rats, molecular biology, and cell culture while Dr. Weigel adds expertise in protein biochemistry and cellular signaling. Dr. Smith's and Dr. Weigel's laboratories are adjacent to each other. They have been collaborating on actions of agonists in bone for six years, hold joint laboratory meetings, and interact on a daily basis. Drs. Peterson and Luo are both located at Baylor College of Medicine, while Dr. Suva is located at the University of Arkansas for Medical Sciences. There will be no difficulty in exchanging bone samples and data with Dr. Suva. Collectively, the investigators have all of the expertise to assess the value of these countermeasures. In these studies we will comprehensively examine the consequences of skeletal unloading and the impact of our countermeasures not only for the desired outcomes of bone density and strength, but at the molecular and cellular levels. This will lead to a better understanding of the causes of skeletal unloading and the means to prevent bone loss.

RESEARCH AREA:	Bone Loss
PRINCIPAL INVESTIGATOR:	Joseph E. Zerwekh, Ph.D.
ORGANIZATION:	UT Southwestern Medical Center at Dallas
PROJECT TITLE:	Prevention of Microgravity-Induced Stone Risk by KMgCitrate

Project Executive Summary

The formation of a renal stone during space flight may have serious negative effects on the health of the crewmembers and the success of the mission. Ground-based studies, as well as a limited number of space flight studies, have clearly demonstrated an increased risk for kidney stone formation as determined from the composition of the urinary environment. Increased bone resorption raises urinary calcium and the urinary state of saturation with respect to the calcium salts, calcium oxalate and brushite. However, documented changes in other urinary components such as citrate, pH, and magnesium appear to also raise the risk for the formation of not only calcium oxalate and calcium phosphate stones but also uric acid stones as well. Nutritional modifications to counter the tendency toward stone formation might include increased fluid consumption and supplementation with an appropriate nutraceutical that would decrease the risk of stone formation by increasing urinary pH and inhibitor concentrations. The hypothesis to be tested in this project is that potassium magnesium citrate supplementation will attenuate the increased risk for stone formation and diminish microgravity-induced bone loss. This hypothesis will be tested during five weeks of bed rest in normal volunteers through three specific aims: 1) assess the efficacy of supplementation with potassium magnesium citrate (KMgCit) in preventing microgravity-induced increased risk of renal stone formation; 2) assess the efficacy of supplementation with KMgCit in reducing microgravity-induced increases in bone resorption and urinary calcium losses; and 3) evaluate the effect of KMgCit supplementation in averting diminished muscle magnesium and potassium concentrations that may occur during microgravity-induced muscle atrophy.

This study is being conducted as a randomized, double-blind, placebo-controlled trial. As such, it is not currently possible to evaluate the effectiveness of this therapy in reducing stone risk and skeletal bone loss until study completion. Although an interim analysis could be performed, doing so could affect the overall statistical power of the study and necessitate inclusion of additional subjects. Despite this limitation, several key observations have continued to be evident in the second year of this study. For the entire group of subjects, mean urinary pH increased during bedrest, most likely reflective of the alkalinizing effect of KMgCitrate. This notion is also supported by increases in urinary potassium, magnesium, and citrate for the group. Net gastrointestinal absorption of alkali also increased significantly for the group. Overall, these changes resulted in no significant change in urine saturation with respect to the stone forming salts of calcium oxalate or sodium urate. In addition, the saturation of urine with respect to undissociated uric acid did not show any significant change from baseline. It is important to note that the lack of change in urinary calcium oxalate saturation was evident despite a marked and significant increase in urinary calcium concentration during bedrest. This rise in urinary calcium concentration and, to a lesser extent in urinary phosphate excretion, is most likely the result of increased bone resorption during skeletal unloading. This is supported by the significant increase in urinary deoxypyridinoline excretion during bedrest, a marker of increased bone resorption. This response is further supported by the fall in serum parathyroid hormone.

At present, it is not possible to discern whether KMgCitrate treatment may have attenuated the apparent increase in bone resorption. This will only be able to be addressed upon completion of the study and comparisons between the placebo and KMgCitrate-treated groups performed.

The current findings for the entire group of studied subjects does not deter from the original hypotheses of this study. The group data are in part consistent with the original hypothesis that provision of alkali, as KMgCitrate, would attenuate the increased stone risk associated with spaceflight and its Earth-based counterpart, bed rest. The observed lack of change in urine saturation with respect to calcium oxalate, despite significant increases in urinary calcium during bed rest, supports this notion. It is not yet clear what effect this agent may have on microgravity-induced bone loss since we did observe changes consistent with increased bone loss. Ultimate proof that KMgCitrate attenuated increased bone resorption during bed rest will require a comparative analysis between placebo- and KMgCitrate-treated subjects upon study completion. Finally, we have not yet analyzed muscle biopsies for magnesium and potassium concentrations. We will perform this analysis upon study completion but before the code is broken for the study. This aspect of the study is to determine if KMgCitrate supplementation can prevent atrophy-related loss of muscle magnesium and potassium.

**NSBRI RESEARCH PROGRAM
CARDIOVASCULAR ALTERATIONS**

Team Leader: Cohen, R. J. MIT

**Associate Team
Leader: Shoukas, A. A. Johns Hopkins**

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RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Mohamed A. Bayorh, Ph.D.
ORGANIZATION:	Morehouse School of Medicine
PROJECT TITLE:	Possible Countermeasures to Post-Suspension Hypotension in the Head-Down Tilt Rat Model
END DATE:	07/31/2004

Project Executive Summary

Exposure to microgravity or simulated microgravity in humans causes cardiovascular deconditioning with orthostatic hypotension and tachycardia. Post-flight orthostatic intolerance is a dramatic physiologic consequence of human adaptation to microgravity made inappropriate by a sudden return to normal gravity. Loss of appropriate cardiovascular reflexes contributes to the cardiovascular deconditioning, but the specific mechanisms remain uncertain. The endothelium is now recognized to play a critical role in the regulation of vascular resistance and blood pressure through the release of nitric oxide and/or prostacyclin. The objective of the proposed studies is to test the hypothesis that the post-suspension hypotension in rats following simulated microgravity involves elevated levels of prostacyclin and/or nitric oxide and, thus, can be attenuated by specific inhibitors of these vasodilatory factors. Using the 30° tail-suspended (hindlimb-unloaded) rat model, the roles of prostacyclin and nitric oxide in post-suspension hypotension are being evaluated. For the coming year we will continue to examine gender differences in the post-suspension hypotensive response.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Donald M. Bers, Ph.D.
ORGANIZATION:	Loyola University Chicago
PROJECT TITLE:	Integrative Cardiac Myocyte Model: Ion Channels, Ca and Contraction
END DATE:	07/31/2004

Project Executive Summary

Specific Aims

- Develop a more up-to-date electrophysiological model of cardiac myocytes.
- Incorporate new Ca transport data on SR Ca uptake, release and Na/Ca exchange.
- Extend the model to include cooperative Ca-dependent contraction and relaxation.
- Implement model in highly accessible computational formats.

Accomplishments

Up-to-date electrophysiological model.

1. We have completed the first major version of our user-friendly computational model of cardiac ion currents and Ca regulation (LabHEART 4.7) and published a manuscript describing it in the *American Journal of Physiology* (Puglisi and Bers). We have made this model freely available for download from our Web site (<http://www.lumc.edu/physio/bers>). Over 500 people have downloaded the program (in 38 countries) and it has already been used successfully in teaching medical and Ph.D. students.
2. We used this model to determine the relative contributions of two key factors contributing to arrhythmogenesis in heart failure (upregulated Na/Ca exchange and downregulated inward rectifier potassium current, I_{K1}).
3. We have added versions for epi-, mid- and endocardial ventricular myocyte which helped a collaborating group (Dr. Andrew McCulloch) to incorporate our cellular model into there more integrative whole heart model.

New Ca transport version of model.

We have updated LabHEART 4.7 model in several ways already.

1. We have further updated ionic currents (e.g. including important characterization and subdivision of transient outward currents, $I_{to,f}$ & $I_{to,s}$; LabHEART 4.9x).
2. We have added the facility that the user can modify the equations that describe the ionic currents and transporters (LabHEART 5.0).
3. We have also overhauled the model (Shannon-model) to include a more appropriate cellular geometry and compartments based on experimental data (including junctional cleft and subsarcolemmal compartments) and used more up-to-date experimentally tested expressions for Ca current, SR Ca release, SR Ca-ATPase and Na/Ca exchange. This major revision is currently being written up for publication and also being ported to a more versatile computational format (from that in which it was developed).
4. We have used this new model to help distinguish the relative importance of 3 factors that contribute to reducing SR Ca content in heart failure: a) reduced SR Ca-ATPase, b) increased Na/Ca exchange function and c) increased diastolic SR Ca leak, each of which we have measured experimentally. This work will be published in *Circulation Research* in October (Shannon, Pogwizd and Bers).

Extend model to include myofilament properties.

1. In parallel with the above, we have developed a novel cardiac myofilament model that includes realistic representations of the steep cooperative force- $[Ca]_i$ relationship, the length-dependence of myofilament activation and the load-dependence of contraction duration. This used local filament nearest-neighbor interactions and Monte Carlo simulations.
2. This work was written up and published as a full paper in the *Biophysical Journal* (Rice and deTombe).
3. This sort of Monte Carlo simulation is not practical for incorporation into a cellular ion channel-Ca transport model. So, we have developed a novel ODE (ordinary differential equation) version of this model which retains reasonably well the important characteristics. This version should be practical to incorporate into our current ion channel-Ca transport model.

Highly accessible computational formats.

This has been an ongoing thrust in all of the above aims.

1. LabHEART 4.7 is the prototype in user friendly version of the model for both teaching and for use by other scientists in the field. The subsequent LabHEART versions have retained this focus (and we have even developed a student tutorial guide).
2. Our work in dovetailing our model for incorporation into McCulloch's whole heart model constitutes another kind of accessibility that is important (but differs from the stand alone LabHEART).
3. Our newer Shannon-model with additional compartments is also currently being developed both ways (flexible for integration in larger scale models, but also for the stand-alone cellular model).

Research Plans

In the final year we will need to complete many of the ongoing modeling efforts, publish manuscript where appropriate and use them in additional ways. Some key aims are to:

- Complete and publish LabHEART 4.9x and 5.0 versions and make them freely available.
- Complete and publish the new Shannon-model, as well as use it to more fully explore how perturbations in conditions (including rate, adrenergic state) alter electrophysiological and Ca handling properties. Additional perturbations are directly related to ongoing studies by Dr. Beverly Lorell's group where changes in expression of Ca transport and ion channels that occur upon cardiac unloading can be more realistically simulated.
- Connect the Shannon-model to the myofilament ODE model to allow the first up-to-date model combining ion channels, Ca transport and contractile elements (in both variants of user friendliness).
- Extend our collaboration with the whole heart modeling efforts of McCulloch's group which will allow more direct studies of the acute affects of cardiac unloading (as in weightlessness) can be explored (and then observed altered cellular expression of transporters and channels) can be superimposed to simulate more long-term systemic compensations.

Countermeasure Development Plans

This particular project is more tuned to providing a computational platform on which to better understand how changes that occur during spaceflight at the more cellular and molecular level can be understood (and intervened upon) in a more integrated framework. In particular the alterations in expression and function with cardiac unloading described by Lorell's group can be incorporated into our computational model (especially when synthesized into the whole heart

context by McCulloch's group) to understand why function is altered and how that may be practically counterbalanced (e.g. by α -adrenergic stimulation or other strategies).

Collaborations

Our group already included collaboration of investigators at four different institutions with complementary strengths (Bers and Puglisi, Loyola University, Chicago; deTombe and Solaro, University of Illinois, Chicago; Shannon at Rush University, Chicago; and Rice, IBM, New York). This allowed good progress to be made along all the specific aims originally proposed. Inter-group collaborative relationships also developed, especially strongly between our group and that of McCulloch, and that has extended the sphere of expertise and impact of both groups with respect to modeling. Additional interactions with Lorell's have brought some of the biological consequences more clearly into view, and minor interactions have occurred with other modeling and experimentally focused teams.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Vincent M. Cassone, Ph.D.
ORGANIZATION:	Texas A&M University
PROJECT TITLE:	Microgravity and Circadian Cardiovascular Function
END DATE:	05/31/2004

Project Executive Summary

This project is directed at the physiological mechanism(s) by which the mammalian circadian clock located within the hypothalamic suprachiasmatic nuclei (SCN) regulates cardiovascular function and to what extent simulated microgravity affects circadian variation in cardiovascular function. The interaction of circadian organization and other determinative factors involved in problems associated with microgravity and cardiovascular disease will be assessed through the comparative study of circadian regulation of cardiovascular function in male vs. female rats.

It is known that astronauts suffer many disruptions to normal bodily processes while in space. The most obvious of these is the redistribution of fluids in the body. This was demonstrated as early as the Mercury era, when man first ventured into space. In the microgravity environment, fluids tend to move into the chest and head, causing facial swelling and congestion. This fluid shift also reduces circulating blood volume and plasma levels of norepinephrine as well as causing a specific increased sensitivity of beta-adrenoreceptors. These changes occur due to a rise in blood pressure as perceived by the carotid baroreceptors. In Earth-based studies, bed-rest with head oriented below the feet (HDT) is believed to simulate these effects in space. HDT causes an attenuation of blood pressure rhythmicity, causing damping out of the circadian rhythm of diastolic blood pressure. Systolic blood pressure was not affected as greatly by HDT. However, HDT did not affect the circadian variation in heart rate. However, studies monitoring heart rate while in flight show that heart rate tends to increase after several days in the microgravity environment. While the circadian period of heart rate may not change, there seems to be an increase in heart rate itself.

a) Specific Aim #1: Determination of SCN Efferents Controlling Circadian Variations of Cardiovascular Function in Long-Term, Conscious Rats: Since it is well-known that cardiovascular responses to pressors and stress are significantly different in anaesthetized vs conscious preparations, we will characterize circadian variation in cardiovascular function in conscious freely moving rats. We will then determine whether surgical blockade of SCN efferents affects the circadian variation of heart rate, cardiac output and mean arterial pressure.

b) Specific Aim #2: Role of Circadian System on Daily and Circadian Variation in Regional Blood Flow: We will determine daily and circadian variations in regional blood flow measurements using ⁸⁵Sr-labelled microspheres in rats whose circadian phases will be monitored independently. We will also determine whether 1) the SCN, 2) SCN efferents and 3) sympathetic innervation are required for the expression of these rhythms.

c) Specific Aim #3: Effects of Simulated Microgravity on Circadian Cardiovascular Rhythms: We will determine the effects of hind-limb unloading on the circadian variation in heart rate, regional blood flow and other cardiovascular variables. Based upon data obtained in Specific Aims #1 and 2, we will determine the mechanisms by which anticipated changes occur. These experiments will provide guidelines for future counter-measures in space.

d) Specific Aim #4: Effects of Gender on Circadian Changes in Cardiovascular Function and Their role in Responses to Microgravity: Because it is well-established that female and male astronauts experience a different set of cardiovascular responses to microgravity, we will also determine whether we can simulate those differences in our simulated microgravitational apparatus. If so, we will employ the information gained in Specific Aims #1 and 2 to provide guidelines for future countermeasures.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Richard J. Cohen, M.D., Ph.D.
ORGANIZATION:	Massachusetts Institute of Technology Harvard-MIT Division of Health Sciences and Technology
PROJECT TITLE:	Cardiovascular Effects of Simulated Microgravity in Man
END DATE:	02/29/2004

Project Executive Summary

This project is targeted towards studying and developing countermeasures to two of the Cardiovascular Critical Risks:

- (i) Development of post-flight orthostatic intolerance
- (ii) Increased susceptibility to ventricular dysrhythmias.

The development of orthostatic intolerance is a well known adverse effect of space flight on the cardiovascular system, and is a current operational problem for NASA. Astronauts post-flight may experience a drop in arterial pressure upon adopting the upright posture after flight, which may be sufficiently severe to cause presyncope or syncope. This effect is greater the longer the duration of the flight, and is more pronounced in women than in men. During space flight intravascular volume is decreased and cardiovascular reflexes are down-regulated because the cardiovascular system is no longer subjected to the stresses associated with changes in posture. Upon return to a gravitational environment, blood pools in the large veins of the lower extremities and the splanchnic circulation, leading to a drop in preload to the heart leading to a decrease in cardiac output. In addition, the reflex ability to increase heart rate and constrict arteries and veins is diminished, and there are also changes in cardiac systolic and diastolic function. Countermeasures of salt and water loading prior to re-entry and the use of G-suits are not adequate countermeasures to prevent the development of orthostatic intolerance, particularly after long duration flights. Our goal with respect to this cardiovascular risk is to better understand the detailed mechanisms leading to orthostatic intolerance and to develop and test mechanism-based countermeasures.

There have been several anecdotal reports of documented episodes of self-terminating ventricular tachycardia during space flight¹⁴. In addition it has been reported that Russian cosmonauts have suffered from ventricular arrhythmias, and two primates have suffered cardiovascular collapse after return from space flight (without ECG documentation). These data suggest that space flight may be conducive to the development of ventricular arrhythmias. However, it is not known whether or not this is in fact the case. If long duration space flight does increase the risk of potentially lethal ventricular arrhythmias then this would obviously pose an enormous problem for very long duration flights. Our goal in this project is to determine in controlled ground-based experiments, whether there is evidence that simulated micro-gravity in fact alters cardiac electrical activity in a manner that may increase susceptibility to ventricular arrhythmias. If we find evidence that this is in fact the case, then we will attempt to establish mechanism and identify potential countermeasures.

In this project we analyze data from ground-based human studies in which 16 day head down tilt bed rest is used to simulate microgravity. We will be studying the following groups in order to examine the effects of gender and age on these cardiovascular risks.

- i. men under age 50
- ii. premenopausal women
- iii. men over age 50

In addition to the effects of bed rest we will also examine the effects of sleep deprivation (another condition of space flight). We will also evaluate the effects of the alpha-agonist midodrine as a countermeasure to the development of orthostatic intolerance. We may also evaluate a countermeasure to the development of ventricular arrhythmias.

The key technologies we will utilize are Cardiovascular System Identification (CSI) as a noninvasive means of assessing closed-loop cardiovascular regulation, and measurement of microvolt T-wave alternans (MTWA) as a noninvasive measurement of changes in cardiac repolarization which has been shown in clinical trials to be an accurate measure to susceptibility to ventricular arrhythmias.

To date in this project, we have found that CSI measures of autonomically mediated cardiovascular reflexes are diminished by bed rest, and that during bed rest there appears to be a shift towards increasing sympathetic/parasympathetic balance. We have found that pre-bedrest CSI measures of increased sympathetic/parasympathetic balance identify those subjects who tolerate tilt both pre-bedrest and post-bedrest. We have put considerable effort into improving CSI technology for use in these studies and for application in biomedical research and patient monitoring.

We have demonstrated that the alpha-agonist midodrine appears to be an effective countermeasure to the development of orthostatic intolerance after exposure to 16 days of simulated microgravity.

We have found that even 16 days of bed rest tends to induce sustained MTWA although not at a level that would be of immediate clinical concern. This evidence does indicate that bed rest does measurably alter cardiac repolarization processes, and raises the issue of whether long term exposure to microgravity could increase susceptibility to ventricular arrhythmias. We have also recently conducted a clinical study that demonstrates that MTWA is an accurate predictor of susceptibility to ventricular arrhythmias and sudden cardiac death in patients with heart failure and no prior history of sustained ventricular arrhythmias.

We plan to continue our ground-based studies in the above identified patient groups, and evaluate the effects of sleep deprivation and midodrine countermeasures. We may also test a proposed countermeasure to the development of ventricular arrhythmias pending future results from these studies. In addition, we have submitted a flight proposal to measure CSI and MTWA pre and post flight, and to evaluate the midodrine countermeasure during flight conditions.

We plan to develop further the CSI and MTWA technologies for use on earth for biomedical research and clinical applications.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Richard J. Cohen, M.D, Ph.D.
ORGANIZATION:	Massachusetts Institute of Technology Harvard-MIT Division of Health Sciences and Technology
PROJECT TITLE:	Effects of Simulated Microgravity on Cardiovascular Stability

Project Executive Summary

This investigation will evaluate and test countermeasures to the development of microgravity-induced orthostatic intolerance and susceptibility to ventricular dysrhythmias. We will apply the Cardiovascular System Identification (CSI) technique for assessing closed-loop cardiovascular regulation and Microvolt T-Wave Alternans (MTWA) technique for assessing susceptibility to ventricular dysrhythmias. The specific aims of the project are:

1. To test a panel of countermeasures to orthostatic intolerance in subjects intolerant to pre-bed rest head-up tilt, in conjunction with the evaluation of CSI and other cardiovascular measures as a potential means of predicting orthostatic intolerance and countermeasure effectiveness.
2. To test in a 16-day, head-down tilt bed-rest study the effectiveness of the orthostatic intolerance countermeasure identified for each individual during pre-bed rest screening, in conjunction with the evaluation of CSI and other cardiovascular measures as a means of predicting orthostatic intolerance and countermeasure effectiveness.
3. To utilize MTWA analysis, in older men and post-menopausal women, to measure the effects of a panel of countermeasures for reducing cardiac electrical instability, and to study the effects of these countermeasures on baseline orthostatic tolerance and closed-loop cardiovascular regulation as measured by CSI and other cardiovascular measures.
4. To test utilizing MTWA analysis in a 16-day, head-down tilt bed-rest study of the effectiveness of the cardiac electrical instability countermeasure identified for each individual during pre-bed rest screening.
5. To further develop and enhance the methodology of CSI, MTWA and plethysmography analyses.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Richard J. Cohen, M.D., Ph.D.
ORGANIZATION:	Massachusetts Institute of Technology Harvard-MIT Division of Health Sciences and Technology
PROJECT TITLE:	Effects of Space Flight on Cardiovascular Stability (Flight Study)

Project Executive Summary

Many astronauts after being weightless in space become hypotensive and presyncopal upon assuming an upright position. This phenomenon, known as orthostatic intolerance, may interfere with astronaut function during reentry and following space flight, and may limit the ability of an astronaut to exit a landed spacecraft unaided during an emergency. Orthostatic intolerance is more pronounced following long-term space flight and is a major concern with respect to the extended flights expected aboard the International Space Station and for interplanetary exploration class missions, such as a human mission to Mars. This problem has also been observed to be more pronounced among women than among men. In addition to the problem of post-flight orthostatic intolerance, a variety of heart rhythm disturbances have been observed in astronauts during and after space flight. The potential lethal arrhythmic risk for astronauts is sustained ventricular tachycardia or ventricular fibrillation, while non-sustained ventricular tachycardia could cause syncope.

In previous ground-based bed rest studies sponsored by NSBRI we have applied two new techniques that we have developed to study the effects of simulated microgravity on the cardiovascular system. Cardiovascular system identification (CSI) has been used as a non-invasive means of measuring alterations in closed-loop cardiovascular regulation and the measurement of microvolt level T wave alternans (TWA) has been used as a non-invasive measure of susceptibility to ventricular arrhythmias. We have also successfully tested the alpha-1 sympathetic agonist midodrine as a countermeasure to the development of orthostatic intolerance. We have found that 16 days of bed rest results in altered cardiovascular regulation. In particular, we have demonstrated alterations in baroreceptor sensitivity, altered electrical stability of the heart, and that midodrine is an effective countermeasure to the development of orthostatic intolerance.

In this proposal we plan to apply the same measurement techniques of CSI and TWA to astronauts pre- and post-flight and to test midodrine as a countermeasure to the development of orthostatic intolerance. This study will allow us to determine if the changes in cardiovascular regulation and cardiac electrical stability measured in a ground-based model also occur during actual space flight. In addition we will test for the first time a potentially highly effective countermeasure for the development of post-flight orthostatic intolerance.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	James E. Coolahan, Ph.D.
ORGANIZATION:	Johns Hopkins University Applied Physics Laboratory
PROJECT TITLE:	Distributed Simulation of Integrated Human Function
END DATE:	04/30/2004

Project Executive Summary

Specific Aims:

1. To develop, at JHU, an experimentally-based computational model of the human ventricular myocyte using cells isolated from tissue biopsies performed in patients; and to develop a finite-element model of the geometry and fiber structure of the human heart using diffusion-tensor imaging data to be collected at JHU, fit by a finite-element model to be developed at the University of California, San Diego (UCSD).
2. To develop a distributed simulation of human cardiac function, incorporating a simulation of the human cardiac ventricular cell resident at JHU based on the model discussed above and a simulation of coupled cardiac mechanical and electrical function resident at UCSD, with distributed simulation control based at JHU/APL;
3. To select other appropriate cardiovascular system models that can be represented over time using simulations, and integrate them into a distributed simulation of cardiovascular function; and
4. To select bone and muscle models that can be represented over time using simulations and integrate them into a multi-function distributed simulation representative of the full integrated human function simulations that will be needed for long-duration space flight.

Main Findings in Reporting Year and Contributions to Answering Critical Questions:

In the Winslow lab at JHU, we have been successful in obtaining from the National Disease Registry Institute (NDRI) two normal human hearts from autopsies of human subjects for which the cause of death was un-related to cardiovascular disease..(NDRI provides extensive clinical information on patient health and cause of death, which allows us to screen for hearts that are representative of the normal state.) One of these two hearts has now been imaged at a spatial resolution of 350x350x800um. The diffusion tensor magnetic resonance imaging (DTMRI) data for this heart are being analyzed, and a finite-element model of heart geometry and fiber structure is being developed. We anticipate that reconstruction data on this heart will be available shortly on the website www.ccbm.jhu.edu. Imaging of the second normal heart is planned. This data will greatly enhance the ability to model dysrhythmias as an aid in determining the associated mechanisms and susceptibilities.

At JHU/APL, we have implemented a computationally-efficient Hybrid Cellular Automata (HCA) model of cardiac electrical activation in 3D cardiac muscle with arbitrary local fiber orientations and local conductivity tensors (that incorporate fiber sheet structure). We demonstrated a model for normal cardiac muscle and also models of muscle with altered electrical properties in a human-size schematic model with 20 million HCA elements on a 250 μ m grid, with a left ventricle volume of 105 ml, and a right ventricle volume of 60 ml. We were

able to simulate one second of physiologic time in one hour on a 3-GHz personal computer (and in 5 minutes for a 1.1-million-element model). We created a visualization of the 3D time-varying data using a PC-based volumetric rendering technique, produced a parallelized version of the code for a massively parallel processor implementation and initiated a collaboration with the NASA Ames Research Center High-Performance Computing (HPC) center. This medium-fidelity model will permit a very time-efficient means to model dysrhythmias, as an aid in determining the associated mechanisms and susceptibilities.

In collaboration with NSBRI researchers at the University of Washington, Case Western Reserve University (CWRU), and the Massachusetts Institute of Technology (MIT), we have developed an integrated physiological simulation of the cycle ergometer exercise protocol used by U.S. astronauts since the Skylab program. We have used a building-block approach – where models of key physiological functions were known to exist within the NSBRI community, we employed those models with minimum modifications; where there were gaps, we developed as simple a functional representation as possible. The integrated simulation includes a cardiovascular system simulation (based on MIT's Research Cardiovascular Simulator, RCVSIM), a simulation of local blood flows, a whole-body metabolism simulation (from CWRU) with embedded skeletal muscle energetics, and a respiration simulation. To connect these various components, we have used the High Level Architecture (HLA) standard for enabling simulation interoperability - originally developed in the U.S. Department of Defense and now an IEEE standard. Each integrated simulation component ("federate" in HLA parlance) executes on a different personal computer, all connected using a local area network. As a part of the exercise simulation effort, we collaborated with John A. Rummel, Ph.D., Deputy Director SA/Space and Life Sciences, NASA/JSC, in recovery of the NASA/Skylab Whole-Body Algorithm (WBA) code. Dr. Rummel provided a 20-year-old magnetic tape of archived WBA FORTRAN code, which we recovered to stable disk storage and returned to Dr. Rummel. We performed a preliminary assessment of the feasibility of future incorporation of a modified version of the WBA respiratory model in our exercise federation.

As a test case, we executed the federated simulation using cycle ergometer data previously collected in a laboratory setting at CWRU. We have also compared the values of heart rate generated by the simulation with those recorded during the exercise tests at CWRU on each of two subjects. Preliminary results show differences between the simulated and measured heart rates of between 5 and 15% during the 15-minute active exercise period. The simulation provides a first step in better understanding the multi-system physiologic responses during the astronaut exercise protocol that has been in use for over 25 years, which can be built upon in developing the improved countermeasures and in understanding the multi-system impact of cardiovascular alterations.

Unique Claims of Study:

The two heart reconstruction data sets from DTMRI will be the first examples of full reconstruction of the fiber organization of the human heart ever achieved.

The 3D HCA heart model provides a means of simulating cardiac electrical activation that executes sufficiently quickly to permit studies spanning minutes of physiologic time duration for

a variety of conditions representing the possible altered electrical physiology associated with prolonged exposure to microgravity. We specifically investigated abnormalities that manifest as Q-T interval prolongation on the electrocardiogram and used this model to explore mechanisms connecting QT-interval prolongation to T-Wave alternans, which in a clinical setting can be a potent predictor of risk of ventricular arrhythmia, if detected at low heart rates during exercise stress testing.

The HLA-based federated simulation of human exercise is the first known computer-based simulation of the cycle ergometer exercise protocol used by U.S. astronauts. It also represents the first demonstration of DoD-developed interoperable simulation technology applied to a significant biomedical research application.

Earth-Based Applications of Research Project:

Both the DTMRI-imaged reconstructed human heart model and the 3D HCA model of cardiac electrical activation have application to studies of dysrhythmias in Earth-based applications. The multi-system physiological simulation of human exercise can be adapted to other Earth-based exercise protocols involving a work rate stimulus.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Michael D. Delp, Ph.D.
ORGANIZATION:	Texas A&M University
PROJECT TITLE:	Circulatory Remodeling with Simulated Microgravity
END DATE:	09/30/2004

Project Executive Summary

Research Program Structure and Design

The proposed studies were designed address the effects of simulated microgravity on the arterial and lymphatic portions of the circulation. Using the hindlimb unloaded rat as a ground-based animal model, the general aim of this proposal is to determine the effects of simulated microgravity on 1) the molecular mechanisms mediating structural remodeling of the arterial resistance vasculature, and 2) the ability of the lymphatics to generate and modulate lymph flow

Specific Aim 1:

To identify early regulatory events leading to hypertrophic remodeling of cerebral arteries in response to hindlimb unloading. We will characterize indicators of cell proliferation to determine whether increased medial thickness previously reported is due to cell growth. Utilizing RT-PCR, in situ hybridization and immunohistochemistry, we will identify key signaling mediators that are involved in this process. Our initial studies will concentrate on nitric oxide (NO) and endothelin (ET), both of which have been proposed to mediate mechanically induced vascular remodeling in other systems. Key cell growth, survival and differentiation markers of vascular smooth muscle cells will be examined to better determine their role in hypertrophic remodeling of the cerebral arteries.

Specific Aim 2:

To characterize signaling events leading to atrophy of resistance arteries in the soleus and gastrocnemius muscle in response to hindlimb unloading. Resistance arteries in the soleus muscle have been shown to atrophy in response to hindlimb unloading in a manner that leads to decreases in luminal diameter as a result of circumferential atrophy of smooth muscle cells (i.e., a decrease in muscle length). Intraluminal shear stress has been shown to decrease during the initial unloading period, but as remodeling occurs, shear stress returns to control levels. The goal of this aim will be to determine if there is increased susceptibility to apoptosis in smooth muscle cells with reduced shear, to evaluate nitric oxide synthase (NOS) activity and expression, and to determine if activation of matrix metalloproteinase activity is up regulated and contributes to decreased luminal diameter. In contrast to the apparent shear stress-mediated remodeling of soleus muscle resistance arteries, reduced transmural pressure appears to be the primary stimulus for remodeling of resistance arteries from the gastrocnemius muscle. The hindlimb unloading-induced remodeling of the gastrocnemius muscle resistance arteries does not involve alterations in the vessel diameter, but rather consists of a decrease in media thickness that appears to occur as a result of radial atrophy of smooth muscle cells (i.e., decreased thickness of smooth muscle cells). We will utilize cellular markers of apoptosis to quantify susceptibility to cell death. RT-PCR, in situ hybridization and immunohistochemistry will be used to quantify expression of growth factors, survival factors and contractile proteins in these vessels in response to hindlimb unloading.

Specific Aim 3:

To evaluate the effects of hindlimb unloading on the ability of the lymphatics from different regions of the body to generate and modulate lymph flow, and thus, regulate overall body fluid homeostasis. It has been demonstrated that acute change in the transmural pressure, luminal flow and outflow resistance will modulate lymph contractile activity and lymph flow. Furthermore, it is known that the special contractile characteristics of the lymphatics are reflected in the expression of contractile proteins within the lymphatic muscle cells. Given that hindlimb unloading induces tissue fluid shifts from the lower to the central and upper portions of the body, it is likely that these tissue fluid shifts will differentially alter the normal physical environment of the lymphatics in different lymphatic drainages. Thus, similar to that found in the arterial circulation, we hypothesize that chronic changes in the normal physical environment of the lymphatics (i.e., changes in tissue fluid pressure, lymph pressure and central venous pressure) will alter both the lymphatic contractile function and the expression of contractile proteins within the lymphatic muscle. Specifically, we will evaluate lymphatic contractile function and contractile protein expression from five regions of the body where significant fluid shifts are known to occur in response to microgravity, as well as in tissues where the majority of the body's lymph is produced.

The present proposal is synergistically related to several projects within the cardiovascular team:

1. The current project PI (Michael Delp) will assist the PI of another NSBRI cardiovascular project, Dr. Vince Cassone, with studies to determine the effects of hindlimb unloading on circadian changes in cardiac output and blood flow distribution.
2. The current studies determining the effects of simulated microgravity on the arterial and lymphatic portions of the circulation are complimentary to those of Drs. Artin Shoukas and Dan Berkowitz, who will investigate the effects of hindlimb unloading on the venous portion of the circulation. Thus, these projects will provide a comprehensive investigation of the peripheral circulation in hindlimb unloaded rats.
3. A collaboration between the current project PI (Michael Delp) and the PI of another NSBRI cardiovascular project, Dr. Chester Ray, was established to determine the effects of microgravity and hindlimb unloading on cardiac mass in rats. This project was funded, in part, by both of the current NSBRI grants to these PIs, and the results have been recently published.

Research Program Accomplishments

The initial studies were designed to determine the mRNA and protein expression of endothelial nitric oxide synthase (eNOS) in cerebral and skeletal muscle resistance arteries, which has been proposed to mediate mechanically induced vascular remodeling in other systems (Specific Aims 1 and 2). In the cerebral arteries, eNOS mRNA expression was not different between control and hindlimb unloaded rats after 1, 14 and 28 days of hindlimb unloading. However, eNOS protein levels are significantly depressed in the middle cerebral artery (MCA) after 14 days of hindlimb unloading.

The current results indicate that eNOS may be directly involved in the remodeling of the cerebral vascular hypertrophy induced by hindlimb unloading in rats. These results were presented at the Humans in Space Symposium, and a manuscript is in preparation.

Lymphatic contractile function from the mesenteric lymphatics and thoracic ducts have been tested and characterized from control rats, and the results have been published. Furthermore, the effect of hindlimb unloading has been determined to diminish the contractile function in these lymphatic vessels. More specifically, there is a 50-75 percent reduction in resting tone of lymphatic vessels, a 30-60 percent reduction in phasic contraction frequency of the lymph pump, a 60-80 percent reduction in the strength of phasic contractions of the lymph pump, and a significant reduction in the pressure-sensitive stimulation of the lymph pump. These results of simulated microgravity have been presented at the Bioastronautics Meeting and the Humans in Space Symposium. A manuscript also is in preparation.

One question in the cardiovascular area has been whether microgravity induces cardiac atrophy. We recently reported that results from rats flown for one week on the Spacelab 3 mission demonstrate that cardiac atrophy does not occur with short-term exposure to microgravity in rats (Ray et al. *J Appl Physiol* 91: 1207-1213, 2001). Similarly, we found that neither one week nor four weeks of hindlimb unloading induced cardiac atrophy or altered the peak rate of rise in left ventricular pressure, and index of myocardial contractility (Ray et al. *J Appl Physiol* 91: 1207-1213, 2001). However, there are studies in the literature reporting cardiac atrophy in hindlimb unloaded rats. To determine whether the cardiac atrophy reported in the literature may be related to caloric deficits in some hindlimb unloaded rats, we plotted heart mass as a function of body mass from all studies reporting these variables in the literature. We found that in all cases where cardiac atrophy was reported, there was a substantial corresponding loss of body mass. Therefore, these findings indicate that cardiac atrophy and dysfunction are not adverse consequences of short-term microgravity or long-term simulations of microgravity when body mass is fairly well maintained.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Beverly H. Lorell, M.D.
ORGANIZATION:	Harvard – Beth Israel Deaconess Medical Center
PROJECT TITLE:	Cardiac Unloading: Biologic Mechanisms and Countermeasures for Cardiac Atrophy
END DATE:	02/29/2004

Project Executive Summary

Project Aims

The aims of the project are to determine functional consequences of cardiac remodeling due to microgravitational unloading using Earth-based model of heterotopic transplantation. The following biologic effects of this surrogate model of cardiac unloading will be examined:

1. Effects on adult myocyte contractile function and Ca^{2+} regulation Regulation of myocyte growth and programmed cell death (apoptosis)
3. Identification of human-relevant countermeasures which blunt cardiac atrophy and/or enhance functional cardiac reserve (including alpha-adrenergic agents).

Key Findings

The key findings of the project to date are the following:

- A. Using the heterotopic transplant model of cardiac unloading, we made three observations:
 1. Cardiac unloading modifies contractile reserve of cardiac myocytes, ie, the ability of heart muscle cells to do extra work.
 2. The biologic mechanism is related to a distinct “molecular signature” the expression of Ca^{2+} regulatory genes in the heart.
 3. The changes are related to magnitude and duration of unloading. These observations have direct implications for planning future human studies, and suggest that lessons from short-term spaceflight may not necessarily predict biologic effects of long-term spaceflight in the hearts of astronauts.

This work is in press in *Circulation*, 2003, pending final requested revisions.

- B. Using genetic mouse models, two novel pathways for preservation of cardiac mass and function have been identified:

1. Cyclin-dependent kinase-9 pathway
2. Telomerase reverse transcriptase pathway.

Impact of Findings

The key findings of the project to date directly confirm the hypothesis of specific aim 1: Cardiac unloading *does* affect both cardiac myocyte contractile function and Ca^{2+} regulation.

Proposed Research Plan for the Coming Year

In the coming year, experiments will focus on specific aim 3: Countermeasure development. We will perform studies to test the hypotheses:

1. Cardiac unloading modifies cardiac adrenergic receptor signaling
2. Therapy aimed at α -adrenergic receptor stimulation may serve as a countermeasure for improvement of cardiac reserve of the heart.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Roger G. Mark, M.D., Ph.D.
ORGANIZATION:	Massachusetts Institute of Technology
PROJECT TITLE:	Computational Models of the Cardiovascular System and its Response to Microgravity and Disease
END DATE:	02/29/2004

Project Executive Summary

Experimental studies of the cardiovascular system in humans, and even in laboratory animals, are necessarily limited in scope because of restrictions on the types of measurements that can be made. Often, the true parameter of interest cannot be measured directly so it must be inferred from other measures. Even when appropriate measurements can be made, the cause of a particular observation may not be evident because of the complexity of the interactions between the numerous components of the system. These and other issues can often be addressed more effectively with the aid of a computational model that simulates the critical components and behaviors of the cardiovascular system. Models depend upon experiments for refinement and specification of their parameters, but also illuminate and enhance the interpretation of experimental results. We view the experiments and computational models as highly synergistic in that the value of one is greatly enhanced by the existence of the other. It could be argued that these are not merely advantages, but essential aspects of a study of orthostatic intolerance.

The primary objectives of this project are to develop a general, modular model of cardiovascular function that contains the essential features associated with the effects of gravity, and to use this model to examine the short term effects of changes in posture before and after exposure to the microgravity environment. The objective of the currently funded project was to extend a previously developed computational model of the cardiovascular system and to use this model to investigate the short-term (0--5 mins) beat-to-beat hemodynamic response of the cardiovascular system to abrupt orthostatic stress --- such as head-up tilt (HUT) or lower body negative pressure (LBNP). The project aimed at facilitating the understanding of the physiology and treatment (prevention) of orthostatic intolerance in post-flight astronauts.

We proposed to:

- Enhance the current version of the cardiovascular simulation to better represent the short-term effects of abrupt orthostatic stress
- Verify the model and use it to investigate and evaluate various hypotheses of orthostatic intolerance, and predict the effects of countermeasures, requiring extensive collection and archiving of experimental data from collaborators.
- Complete, document, and disseminate to other investigators a form of the model implemented in JAVA
- Apply the cardiovascular model to the clinical problem of intelligent patient monitoring with particular emphasis on establishing an enhanced research database of multiparameter hemodynamic data from intensive care patients.

Earth-Based Applications of Research Project

While the primary purpose of the proposed modeling effort is to provide a tool for use in studies of orthostatic hypotension and countermeasures, the resulting models will have value in other settings as well. As a clinical tool, models enhance the quality of measurement, both in the context of remote clinical monitoring in the space environment and in the ground-based clinical setting. Continuous hemodynamic monitoring in the ICU could be augmented by the use of models capable of tracking a particular patient's status. The challenge of rapidly assessing the pathophysiologic state of an unstable patient is made more difficult as a result of the density of the available data, and its frequent lack of logical and coherent organization. Quantitative hemodynamic models of the type developed here can provide a rational structure around which to present the data to clinicians, providing the basis for more sophisticated and sensitive alarms, and playing a pivotal role in developing decision support paradigms to guide therapy. Computational models of cardiovascular function are also valuable educational tools for students in high school, college, university, and medical school.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Roger G. Mark, M.D., Ph.D.
ORGANIZATION:	Massachusetts Institute of Technology Harvard-MIT Division of Health Sciences and Technology
PROJECT TITLE:	Computational Models of Cardiovascular Function for Simulation, Data Integration and Clinical Decision Support

Project Executive Summary

One of the highest priority problems in the current manned space program is orthostatic intolerance (OI) experienced by astronauts upon their return to the normal gravitational environment. This problem has been well known since the earliest days of manned space flight and has been intensely studied inflight and in many ground-based (bed-rest) studies. A number of countermeasures have been proposed and evaluated, but to date, no effective and practical countermeasure has been developed. While the effectiveness of countermeasures can only be explored in ground-based experiments and ultimately be proven successful when used by astronauts, the small number of subjects studied in both situations makes the interpretation of limited experimental data for countermeasure development and refinement difficult.

Mathematical models that represent the essential functional components of the cardiovascular system provide a powerful means to extract physiologically relevant information from multiparameter experimental data.

We will extend progress already made, with the following specific aims:

1. Develop procedures and algorithms for automated model-based extraction of physiologically relevant information from multivariate data streams, particularly those relevant to countermeasure development for OI. We will expand our unified data archive of hemodynamic signals during gravitational stress. Our model-based methodology will provide a basis for data integration and interpretation, and will support the generation and evaluation of physiologically meaningful hypotheses.
2. Develop strategies for systematic and effective model identification, i.e., for the adaptation of model structures and parameters to the characteristics of the available physiological data. These strategies will be based on analytical and computational studies of candidate models and their sensitivities to parameter changes.
3. Apply our computational modeling and parameter identification technology to the challenge of cardiovascular monitoring of critically ill patients. Hemodynamic measurements will be transformed into physiologically meaningful parameters through model-based processing.
4. Develop and thoroughly test a graphical user interface for our current cardiovascular model. The resultant cardiovascular simulator will be open-source and capable of running on all major operating systems. Furthermore, the entire model will be made publicly available to researchers and educators.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Andrew D. McCulloch, Ph.D.
ORGANIZATION:	University of California, San Diego
PROJECT TITLE:	Integrated Modeling of Cardiac Mechanical and Electrical Function
END DATE:	07/31/2004

Project Executive Summary

Project Aims

Aim 1: To apply our existing techniques for modeling three-dimensional cardiac mechanics and action potential propagation to develop anatomically detailed three-dimensional dynamic finite element models of regional cardiac electromechanics.

Aim 2: To bridge models and data on cardiac metabolism and cellular dynamics with systems models of coronary flow, central hemodynamics, and cardiovascular regulation.

Aim 3: To develop tools for using available wall motion data from medical imaging in man to validate the mechanoenergetic models and identify myocardial constitutive properties.

Aim 4: To apply new models of geometric and constitutive remodeling in response to chronically altered external loading conditions to develop simulations of long-term cardiac adaptation to microgravity.

Aim 5: To implement the models using modular object-oriented software engineering techniques that allow the models to be readily integrated with others through standard broker architectures for software interoperability.

Aim 6: To collaborate with other prospective projects in the Integrated Human Function Core.

Key Findings/Progress

Significant progress has been made coupling cardiac electromechanical models. Two new papers have been accepted applying and extending the a new computational model reported last year (Usyk TP, LeGrice IJ, McCulloch AD. Computational model of three-dimensional cardiac electromechanics. *Comput Visual Sci* 4(4):249-257, 2002.) in which a model of anisotropic cardiac impulse propagation is coupled to a model of three-dimensional anisotropic ventricular wall mechanics in an anatomically detailed three-dimensional model of the left and right ventricles that also includes a model of the Purkinje fiber network anatomy. In one new paper now in press (Usyk, T. P. and A. D. McCulloch. Relationship between regional shortening and asynchronous electrical activation in a three-dimensional model of ventricular electromechanics (*Journal of Cardiovascular Electrophysiology* 14 (suppl.) this model has been used to investigate the effects of altered mechanical pacing sequence due to ectopic activation, and showed excellent agreement with published experimental data. In another paper now in press (Usyk, T. P. and A. D. McCulloch. Electromechanical model of cardiac resynchronization in the dilated failing heart with left bundle branch block. *J Electrocardiol*, 2003.), we tested the ability of the model to predict countermeasure effectiveness, by using it to predict the effects of bi-

ventricular pacing for cardiac resynchronization therapy in an experimental model of heart failure with a conduction defect. Again the model predictions showed very good agreement with experimental measurements.

As a result of the NSBRI Exercise workshop in Seattle, models of cardiac electromechanics during exercise are focusing on the effects of heart rate, adrenergic stimulation and acidosis. We have developed a new model of adrenergic signaling in the cardiac myocyte that couples to electrophysiology by simulating the effects of β adrenergic receptor stimulation on excitation-contraction coupling mechanisms via phosphorylation of several cellular targets by protein kinase A. This new paper now in press (Saucerman, J. J., L. L. Brunton, A. P. Michailova, A. D. McCulloch. Modeling beta-adrenergic control of cardiac myocyte contractility in silico. *J Biol Chem*, 2003.) successfully recapitulates major effects of the neurohormonal activation that occurs during exercise on the ventricular myocyte.

A new object-oriented modular version of *Continuity* that was released during the previous period has been updated. A new release, tentatively named release 6.2 is due for release in the Fall 2003. It has a modular client-server design, very high-level scripting language, a graphical user interface and new three-dimensional viewer. The newest version supports additional computer platforms, especially Linux. It has also been used successfully in classroom teaching.

We have engaged in productive collaborative activities with other members of the Integrated Human Function Core, especially the group of Dr. Bers. As the result of discussions with the Cardiovascular Alterations Team, we have conducted preliminary experiments on changes in restitution dynamics with mechanical loading. This will provide a basis for new computational models of mechanoelectric feedback as a follow-up to our recent report showing how altered mechanical loading slows action potential propagation and prolongs repolarization in the whole heart (Sung D, Mills RW, Schettler J, Narayan SM, Omens JH, McCulloch AD. Ventricular filling slows epicardial conduction and increases action potential duration in an optical mapping study of the isolated rabbit heart. *J Cardiovasc Electrophysiol* 14(7):739-749, 2003.)

Impact

These findings of the three-dimensional electromechanical models validate the original premise of the proposal that an integrated cardiac three-dimensional electromechanical model is both computational feasible and physiologically predictive. They also demonstrate the utility of such models for countermeasure design, assessment and validation. This is a fundamental advance that paves the way for the other objectives and applications. By including cell signaling pathways in the cellular models of cardiac excitation-contraction coupling, we will be in a position to simulate the physiological effects of exercise and other stresses that activate the major pathways that respond to neurohormonal signals in the heart.

The new software, *Continuity 6.0* provides a problem solving environment for integrative modeling that is general enough for both structurally integrated models that couple from single cell to organ system scales and functionally integrated models that couple electrophysiology, mechanics, metabolism and regulatory processes. The methods are generic and thus applicable to other systems such as soft tissues and muscle.

Research Plan

We will extend the electromechanical models to include more detailed cellular biophysical models of cardiac excitation and contraction whose parameters therefore have greater

physiological meaning thus making the models more inherently predictive. These cellular models are developed by our group and the group of Dr. Bers including new models of ionic currents, excitation-contraction coupling, myofilament activation and crossbridge interactions. The integrative whole heart models that contain these improved cellular models will also be used to incorporate the effects of regional cellular heterogeneity in the heart walls. For example, the T-wave of the electrocardiogram may be an important predictor of potentially life-threatening cardiac events in space. The morphology of this waveform is directly influenced by the fact that myocyte repolarizing currents are different as a function of transmural position in the ventricular wall. We have therefore begun a new model in collaboration with Dr. Bers lab that includes three transmural cell layers in the epicardium, M-cell layer and endocardial regions of the ventricular walls. We will also extend the current cellular models model to include the pH regulation of intracellular calcium handling, the regulation of cardiac electrophysiology and contraction by beta adrenergic stimulation, and the effects of magnesium which may offer a potential countermeasure. The next major release of *Continuity* will include improved facilities for composing and integrating cellular models and for the efficient parallel solution of large-scale whole organ models. Synergistic collaborations with other NSBRI projects will be continued.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Janice M. Meck, Ph.D.
ORGANIZATION:	NASA Johnson Space Center
PROJECT TITLE:	Mechanisms of Post-Space Flight Orthostatic Intolerance (Flight Study)

Project Executive Summary

There is still a significant number of astronauts at Johnson Space Center who suffer from post-flight orthostatic hypotension and presyncope. The mandatory use of fluid loading with salt tablets and water, anti-gravity suits, and the liquid cooling garment has not eliminated the problem. A growing body of evidence suggests that there are major physiological systems that become dysfunctional as a result of space flight. The degree of dysfunction varies from minimal to severe. Several studies have provided evidence that autonomic function is impaired during and after space flight. Additional factors such as local factors, could also be involved. An area that has not been studied in humans is the effects of space flight on nitric oxide physiology and its modulation of blood pressure. The study proposed in this application will continue the pursuit of mechanisms of autonomic dysfunction in presyncopal astronauts. In addition, it will begin to elucidate changes in nitric oxide production and the resulting effects on the cardiovascular system. This study will not have in-flight measurements. All procedures will be performed before launch, on landing day and three days after landing. The study has two specific aims: 1) to compare pre-flight to post-flight changes in responses of veins to adrenergic agonists between presyncopal and nonpresyncopal astronauts; 2) to compare pre-flight to post-flight changes in nitric oxide levels, inducible nitric oxide synthase messenger RNA and protein, cell adhesion molecules associated with endothelial activation, responses to acetylcholine with and without nitric oxide synthase inhibition, and reactive hyperemia responses in the brachial artery, the arm and the popliteal artery in the leg, between presyncopal and nonpresyncopal astronauts. Presyncopal and nonpresyncopal astronauts will be defined by their ability to complete a 10-minute upright tilt test on landing day.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Ferid Murad, M.D., Ph.D.
ORGANIZATION:	The University of Texas Health Science Center at Houston
PROJECT TITLE:	A Soluble Guanylyl Cyclase Mouse Knock-Out Model

Project Executive Summary

The goal of the proposed study is to generate a soluble guanylyl cyclase (sGC) knock-out mouse model to elucidate the role of sGC in the cardiovascular system under physiological and microgravity conditions. While the role for sGC in the vasculature was first identified 20 years ago, the precise understanding of the physiological significance of sGC-cGMP signaling in the myocardium remains incomplete.

The exposure of astronauts to microgravity conditions in space results in cardiovascular alterations that are characterized by orthostatic intolerance and decreased exercised capacity after their return to Earth and gravity. However, the mechanisms of adaptation to microgravity conditions are unknown.

As nitric oxide and sGC play a significant role in the cardiovascular system, we hypothesize that sGC plays a role in adaptation to microgravity. To investigate this hypothesis, we will use a gene targeted mouse animal model with myocardium-specific and smooth muscle-specific disruption of gene expression to study the specific role of sGC in microgravity/gravity adaptation.

In specific aim 1, a mouse animal model with myocardium-specific and vascular smooth muscle-specific disruption of sGC beta 1 gene will be developed. Following development, we will characterize the effects of myocardial-specific and vascular smooth muscle-specific gene disruption on general appearance, embryonic development, histology of the heart and hemodynamic changes in knockout mice versus wild-type mice.

In specific aim 2, we will determine the role of sGC pathway deficiency on the development of orthostatic intolerance that occurs during re-adaptation to gravity using the established tail-suspended rodent model to stimulate the microgravity conditions. The time course of changes in cardiovascular function induced by re-adaptation to gravity in knock-out mice versus wild type mice will be studied. Finally, the cardiovascular responsiveness in knock-out mice versus wild-type mice following de-suspension will be assessed using vaso- and cardio-active agents. Comparisons between knockout mice and wild-type mice in simulating weightlessness conditions will aid to identify sGC- dependent pathways necessary for adaptation to microgravity and re-adaptation to gravity.

In order to fulfill specific aim 1, we are developing a mouse animal model with myocardium-specific and vascular smooth muscle-specific disruption of sGC gene. Last year, we used Lox-Beta 1 sGC targeted vector generated in the previous year to produce the gene-targeted mouse ES line. Targeted ES cells were introduced into mouse blastocysts and chimeric

male mice were obtained. In order to identify animals with a transmission of the targeted ES cells into the germ line chimeric males were bred with wild-type females.

Presently, we are at the stage of genotyping F1 offsprings to identify Lox-targeted heterozygous mice. Homozygotes with Lox-targeted *Beta1* sGC gene will be obtained by crossing identified heterozygotic mice. In the upcoming year, *Beta1* sGC-lox mice will be bred with α -MyHC-Cre mice containing a myocardium specific Cre-recombinase, to generate myocardium specific *Beta1*-sGC-Cre/lox knockout mice and [SM-CreER(T2)(ki)] containing smooth muscle-specific Cre recombinase to produce smooth muscle-specific *Beta1*-sGC-Cre/lox knockout mice. Following their development, we will characterize the effects of myocardial-specific and vascular smooth muscle-specific gene disruption on general appearance, embryonic development, histology of the heart and hemodynamic changes in knock-out mice versus wild-type mice.

Earth-Based Applications of Research Project

sGC is the heterodimeric enzyme that catalyzes the production of cGMP from GTP. In vivo, sGC represents a major receptor for nitric oxide (NO), a unique signaling molecule with numerous physiological functions, including relaxation of smooth muscle, inhibition of platelet aggregation and modulation of cellular differentiation. The cardiovascular functions of sGC that are mediated via activation by NO, together with the particulate isoform of guanylyl cyclase, activated by atrial natriuretic peptide (ANP), are important in the regulation of cardiovascular and renal function.

The NO-sGC pathway affects cardiac performance by influencing myocardial contractility, chronotropy and energy production. cGMP produced by vascular smooth muscle sGC in response to NO generated by the vascular endothelium is a major component of vasodilatory signaling pathways in coronary arteries of the heart and other vessels. While the role of sGC in the vasculature was first identified 20 years ago, the physiological significance of sGC-cGMP signaling in the myocardium is not yet fully understood. Indeed, the significance of cGMP signaling in cardiomyocytes is controversial with conflicting reports in literature.

Several problems are associated with the study of sGC-mediated physiology. Distinctions between cGMP-dependent and -independent actions of NO and between the physiological contributions of cGMP produced by the particulate and soluble forms of the enzyme are difficult to determine due to the lack of selective sGC inhibitors. Furthermore, at least two sGC isoforms demonstrate very similar pharmacological and functional properties. A more precise understanding of sGC-cGMP signaling and regulatory events would have profound pathophysiological significance and could potentially help to develop novel therapeutic strategies with minimized negative side effects. A gene-targeted mouse model represents a unique opportunity to study sGC-cGMP action and to clarify the role of sGC in these processes.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Chester A. Ray, Ph.D.
ORGANIZATION:	Pennsylvania State University
PROJECT TITLE:	Effect of Simulated Microgravity on the Vestibul sympathetic Reflex in Humans
END DATE:	08/31/2004

Project Executive Summary

Original Aims:

1. To determine MSNA responses to head-down neck flexion (HDNF) before and after 1 and 7 days of HDBR. HDNF has been used in our laboratory to activate the vestibular system (i.e., otolith organs) in humans. We have shown that HDNF elicits increases in MSNA. We hypothesize that MSNA responses to HDNF will be attenuated after HDBR and that the attenuation of MSNA will increase as a function of HDBR duration. If the hypothesis is true, this would be the first evidence that vestibular system may participate in the regulation of MSNA after simulated microgravity (i.e., HDBR) and possibly spaceflight.
2. To determine MSNA responses to HDNF during lower-body negative pressure before and after HDBR. We have shown that MSNA is augmented by HDNF during lower-body negative pressure. Thus in healthy adults, the vestibul sympathetic reflex can help defend against orthostatic challenges by increasing MSNA. We hypothesize that HDNF after HDBR will not increase MSNA during lower-body negative pressure. Additionally, we hypothesize that there will be an inhibitory interaction between the vestibul sympathetic reflex and baroreflexes. Therefore, after HDBR the vestibul sympathetic reflex will be impaired and not be able to help defend against an orthostatic challenge by increasing MSNA. This finding would give credence to the concept that alterations in the vestibul sympathetic reflex may participate importantly in post-spaceflight OI.

Key Findings:

During this granting period our laboratory has made significant contribution in the area of the vestibul sympathetic reflex in humans and orthostatic intolerance. We have published 25 papers in top ranked scientific journals with additional manuscripts in preparation. We summarize a selection of these results below:

- Effect of Short-Term Microgravity and Long-Term Hindlimb Unloading on Rat Cardiac Mass and Function: These data suggest that cardiac atrophy does not occur following short-term exposure to microgravity, and that neither short- nor long-term simulated microgravity alter cardiac mass or function.

- Effect of Age on the Vestibul sympathetic Reflex: These data indicate that aging attenuates the vestibul sympathetic reflex in humans and may contribute to the increased prevalence of orthostatic hypotension with age.
- The Vestibul sympathetic Reflex During Orthostatic Challenge in Aging Humans: These data provide experimental support for the concept that age-related impairments in the vestibul sympathetic reflex persist during orthostatic challenge in older adults. Furthermore, these findings are consistent with the concept that age-related alterations in vestibular function might contribute to altered orthostatic blood pressure regulation with age in humans.
- Attenuated Sympathetic Nerve Responses After 24 Hours of Bed Rest: These findings suggest that 24 h of bed rest reduces sympathetic nerve responses to LBNP.
- Melatonin Attenuates Sympathetic Nerve Responses to Orthostatic Stress in Humans: These findings indicate that high concentration of melatonin can attenuate reflex sympathetic increases to orthostatic stress in humans. These alterations appear to be mediated by melatonin-induced changes to the baroreflexes.
- Interaction of the Vestibul sympathetic and Arterial Chemoreflexes: These findings indicate an additive neural interaction between the vestibul sympathetic reflex and arterial chemoreflex for MSNA, but an inhibitory interaction on mean arterial pressure and heart rate. Therefore, no central modulation exists between these two reflexes with regards to MSNA output in humans.
- Limb Neurovascular Control During Activation of the Vestibul sympathetic Reflex: These results indicate that there is not differential control of MSNA in the arm and leg during altered feedback from otolith organs in humans, but that greater vasoconstriction occurs in the calf than the forearm. These findings indicate that vasodilation occurs in other vascular bed(s) to account for the lack of increase in arterial blood pressure during HDR.
- Effect of Vestibular Activation on Respiration: These data indicate that semicircular canals, but not otolith organs or neck afferents, mediate an increased respiratory rate in humans and support the concept that vestibular activation alters respiration in humans.
- Aging Attenuates the Vestibul respiratory Reflex in Humans: The results of this study indicate that the vestibul respiratory reflex is attenuated in older humans, with greater vestibular stimulation needed to activate the reflex.
- Vestibul sympathetic Reflex During Mental Stress: We conclude that the interaction for MSNA and arterial pressure is additive during combined vestibular and mental stimulation. Therefore, vestibular- and stress-mediated increases of MSNA appear to occur independently in humans.

- Aging, Opioid Receptor Agonists and Antagonists, and the Vestibulospinal Reflex in Humans: These data do not provide experimental support for the concept that opioids modulate the vestibulospinal reflex in humans. Moreover, endogenous opioids do not appear to contribute the age-associated impairment of the vestibulospinal reflex.

Major Impact:

During this funding period we found that aging may serve as an excellent model for determining the effect of microgravity on the vestibulospinal reflex.

Earth-Based Applications of Research Project

Our studies may provide a new mechanism responsible to orthostatic intolerance in humans. Also, our results provide a rationale for developing a new treatment of orthostatic intolerance in humans via the activation of the vestibulospinal reflex.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Chester A. Ray, Ph.D.
ORGANIZATION:	Pennsylvania State University
PROJECT TITLE:	Ultrasonic Bone Stimulation: Countermeasure to Orthostatic Intolerance

Project Executive Summary

Orthostatic intolerance (OI) is a major physiological consequence of space flight. It has been estimated that nearly one-half to two-thirds of all astronauts experience OI after space flight. Postflight OI is a serious medical issue for manned space flight because physical performance of an astronaut is severely impaired at a time when rapid egress from the spacecraft may be needed during an emergency. A number of physiological factors have been suggested to contribute to this problem with a number of countermeasures developed to prevent or alleviate postflight OI. However, as of yet, no single countermeasure has effectively prevented postflight OI.

The overall objective of this project is to determine whether ultrasonic bone stimulation of the mastoid improves orthostatic tolerance and thus could serve as an effective and simple countermeasure for postflight OI. Based on preliminary data, it is hypothesized that ultrasonic bone stimulation of the mastoid will improve orthostatic tolerance in OI patients and in subjects exposed to bed rest.

A secondary goal of this project is to determine the mechanism by which ultrasonic bone stimulation of the mastoid increases orthostatic tolerance. It is hypothesized that this novel countermeasure acts to increase sympathetic nerve activity through engagement of the vestibulosympathetic reflex. Direct measurement of muscle sympathetic nerve activity will be made during ultrasonic bone stimulation in young, aged, and vestibular-deficient subjects. This project should provide clear information on the possible role of ultrasonic bone stimulation of the mastoid as an effective and simple countermeasure for OI following space flight.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Artin A. Shoukas, Ph.D.
ORGANIZATION:	Johns Hopkins University School of Medicine
PROJECT TITLE:	Mechanics of Cardiovascular Deconditioning
END DATE:	04/30/2004

Project Executive Summary

Specific Aims:

1. To determine mechanisms of impaired stroke volume response (SV) in a rat model of micro-gravity. The impaired stroke volume response seen in HLU rats could result from either functional and/or structural changes in the myocardium. Example a (below) addresses the former and example b addresses the latter.
 - a. To determine the role of myocardial contractility and loading conditions in the impaired SV response. We hypothesized that the impaired SV response is secondary to both altered loading and impaired myocardial contractile reserve.
 - b. To determine the role of cardiac atrophy in cardiovascular de-conditioning. We hypothesized that micro-gravity is associated with a significant loss of cardiac mass.
2. To determine molecular mechanisms of vascular (systemic and pulmonary arterial, and venous) hypo responsiveness in a rat model of micro-gravity. Our data demonstrates impaired contractile responses in both arterial, venous, and pulmonary vascular beds, and an increase in venous capacitance in HLU rats.
 - a. To determine the role of abnormalities in vascular smooth muscle Ca^{2+} influx/release and myo-filament Ca^{2+} sensitivity in vascular hypo-responsiveness. We hypothesized that abnormalities in $[Ca^{2+}]_i$ and/or myo-filament Ca^{2+} sensitivity in smooth muscle may contribute to the endothelial independent mechanism of vascular contractile dysfunction.
 - b. To determine the role of the endothelium in vascular contractile hypo-responsiveness. We hypothesized that enhanced endothelial dependent vasodilator signaling contributes to vascular smooth muscle hypo-responsiveness.
3. To test pharmacologic countermeasures based on mechanisms that impair both SV responses and vascular hypo-responsiveness in a rat model of micro-gravity. We hypothesize those pharmacologic countermeasures that enhance vascular smooth muscle contraction by modulating signal transduction pathways, can be used as countermeasures to treat orthostatic intolerance associated with cardiovascular de-conditioning.

Summary of Accomplishments:

Our proposed experiments test the overall hypothesis that alterations in venous capacitance function and arterial resistance by the carotid sinus baroreceptor reflex system are an important determinant of the cardiac output and blood pressure response seen in astronauts after returning to earth from long term exposure to microgravity. This hypothesis is important to our overall understanding of circulatory adjustments made during long term space flight. It also provides a framework for investigating countermeasures to reduce the incidence of orthostatic hypotension caused by an attenuation of cardiac output. We continue to use hind limb un-weighted (HLU) rat

model to simulate the patho-physiological effects as they relate to cardiovascular de-conditioning in microgravity. We have used this model to address the hypothesis that microgravity induced cardiovascular de-conditioning results in impaired vascular responses and that these impaired vascular responses result from abnormal alpha-1 AR signaling. The impaired vascular reactivity results in attenuated blood pressure and cardiac output responses to an orthostatic challenge.

We have used in-vitro vascular reactivity assays to explore abnormalities in vascular responses in vessels from HLU animals and cardiac output (CO), blood pressure (BP) and heart rate (HR) measurements to characterize changes in hemodynamics following HLU. Overall, we have been able to show that our model of microgravity exposure is associated with a decrease in sympathetic neurotransmission (SN). This in turn is associated with a decrease in alpha-1 AR number and signaling as well as vessel smooth muscle mass (trophic effects of NE). Upon return to gravity, attenuated vascular contractility occurs secondary to end organ hypo-responsiveness, despite normal or accentuated sympathetic neurotransmission. We have found that the impaired venular and arteriolar responses to catecholamine stimulation result in impaired stroke volume, cardiac output and blood pressure responses.

These accomplishments have allowed us to refine mechanisms, begin to test countermeasures - specifically Midodrine - and bridge the gap between animal models and human subjects in our understanding of microgravity-induced orthostatic intolerance.

Earth-Based Applications

These studies have direct application to orthostatic intolerance seen in the elderly population, particularly in females. Similar countermeasures that have been proposed for astronauts in a microgravity environment are also currently being tested in the female elderly population.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Artin A. Shoukas, Ph.D.
ORGANIZATION:	The Johns Hopkins University School of Medicine
PROJECT TITLE:	Non-Adrenergic Mechanisms of Cardiovascular Deconditioning

Project Executive Summary

Cardiovascular remodeling in response to microgravity predisposes astronauts to orthostatic intolerance and decreased exercise tolerance on return to a 1-G environment. It is now appreciated that altered loading of the heart and blood vessels, and neuro-hormonal signals, drive these changes which result in remodeling and cardiovascular deconditioning. While central autonomic processing and efferent sympathetic and parasympathetic responses have been shown to contribute to orthostatic intolerance, end organ hypo-responsiveness has recently been shown to be of critical importance in the deconditioning/hypo-responsive phenotype.

In new data within this grant, we demonstrate that nitric oxide, an important cardiovascular signaling molecule, is an important mediator of cardiovascular deconditioning. We demonstrate:

1. the NO/cGMP pathway is up-regulated and contributes to vascular hypo-responsiveness;
2. myocardial contractile reserve is depressed in cardiac myocytes from animal models of microgravity;
3. the presence of a beta adrenergic receptor (β -3 AR) coupled to NOS-3 that acts as a negative modulator of β -AR mediated enhanced myocardial contractility; and
4. the critical role of the NO/cGMP system in the prevention of myocardial hypertrophy.

We hypothesize that the NO/cGMP pathway is up-regulated in both the heart and vasculature resulting in vascular and myocardial contractile hypo-responsiveness, as well as vascular and myocardial atrophy.

We will determine the role of NO/cGMP system in the vascular hypo-responsiveness, myocardial contractility and cardiac remodeling, as well as the interaction of vessels and heart, ventricular-vascular coupling. Furthermore, based on our findings, we propose novel countermeasures aimed at inhibiting these dysregulated pathways. We will determine:

1. the role of the endothelium and the NO/cGMP pathway in vascular hypo-responsiveness and remodeling in microgravity;
2. the role of NO in modulating myocardial contractility and hypertrophy;
3. whether up-regulation of the NO/cGMP system results in decreased vascular and ventricular stiffness as measured by integrated cardiovascular function and ventricular-vascular coupling; and
4. test countermeasures aimed at inhibiting NO/cGMP pathways for their ability to enhance vascular tone and attenuate vascular hypo-responsiveness, restore myocardial contractile reserve, prevent NO mediated anti-hypertrophic responses, and restore arterial and ventricular stiffness.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Gordon H. Williams, M.D.
ORGANIZATION:	Harvard – Brigham and Women's Hospital
PROJECT TITLE:	Influence of Gender and Age on Renal and Cardio- Endocrine Responses to Simulated Microgravity
END DATE:	02/29/2004

Project Executive Summary

Orthostatic intolerance remains an operational problem following space flight, and has been observed to be more pronounced among women than among men. In addition, there is growing evidence that cardiac dysrhythmias may pose a threat to the health of space travelers.

In our previous studies we observed that subjects on a constant high dietary sodium intake during simulated weightlessness have varying degrees of sodium balance response. Furthermore, the variability in response correlated with orthostatic tolerance and subjects age. The overall goal of this study is to assess in women (a population at increased risk for orthostatic intolerance) and in men over the age of 50 (an age range more consistent to that of astronauts than the <35 year olds involved in our previous studies) the impact of simulated microgravity on volume-regulating systems. A secondary objective is to search for any correlation between changes in these systems and changes in myocardial electrical stability. A final goal is to determine the effect of two potential countermeasures: midodrine in women and low-dose spironolactone in older men.

This work has implications for the treatment and prevention of maladaptive hemodynamic responses experienced by astronauts in flight and on return to Earth. It will increase our understanding of the mechanisms by which weightlessness changes volume and sodium homeostasis, and possibly cardiac electrical stability, thereby, providing entree to develop appropriate countermeasures. Perhaps most importantly, it will broaden our database to include older individuals and women, two groups who are well represented among our population of current and future space travelers. Finally, the results of these studies may further our understanding of the pathophysiology of alterations in volume homeostatic mechanisms in cardiovascular diseases such as congestive heart failure.

Specific Aims:

1. To investigate the influence of age on the pattern of renal sodium handling and the acute responsiveness of the RAAS following simulated microgravity exposure
2. To investigate the influence of gender on the pattern of renal sodium handling and the acute responsiveness of the RAAS following simulated microgravity exposure
3. To investigate the effects of the alpha-1 agonist midodrine, as a countermeasure against orthostatic intolerance following microgravity exposure in women
4. To investigate the effects of spironolactone in older men on the renal-endocrine responses to simulated microgravity, and on changes in myocardial electrical stability resulting from microgravity exposure

Main Findings:

The data we have compiled thus far has shown that individual predisposition plays a very important role in orthostatic intolerance. We found that several baseline physiological characteristics are associated with an increased susceptibility to orthostatic intolerance. These characteristics include, but are not limited to, the renin-angiotensin-aldosterone system, the autonomic system, and leg venous compliance. These findings helps us in answering CQ 3.08 by identifying the critical characteristics that contributes to orthostatic intolerance enabling us to create a new model for the role of predisposition in orthostatic intolerance.

Unique Claims of Study:

Individual predisposition plays a critical role in the development of orthostatic intolerance.

Earth-Based Applications of Research Project:

The results of this study may further our understanding of the pathophysiology of alterations in volume homeostatic mechanisms in cardiovascular diseases such as congestive heart failure.

RESEARCH AREA:	Cardiovascular Alterations
PRINCIPAL INVESTIGATOR:	Gordon H. Williams, M.D.
ORGANIZATION:	Harvard – Brigham and Women's Hospital
PROJECT TITLE:	Effects of Microgravity on Renal, Endocrine and Volume-Regulatory Function

Project Executive Summary

Orthostatic intolerance remains an operational problem following space flight. In addition, there is growing evidence that cardiac dysrhythmias may pose a threat to the health of space travelers. At present, no effective countermeasures exist for orthostatic intolerance and for the probable increased risk of ventricular dysrhythmias.

In the last six years, we have addressed the subject of renal, endocrine and volume-regulating function during head-down tilt bed rest. We have found that simulated microgravity induces changes in the volume-regulating systems and electrolyte excretion, and leads to changes in cardiac electrical stability. We have also found that using our tilt-test protocol, the pre-bed rest tilt-test tolerance was predictive of post-bed rest tilt-test tolerance. We propose now to conduct two double-blinded randomized trials to:

- 1) Investigate the use of a pre-bed rest tilt-test as a means to screen countermeasures against orthostatic intolerance, and investigate the influence of these countermeasures on the renal and endocrine responses to orthostatic stress.
- 2) Investigate the effectiveness of individualized countermeasures identified during a pre-bed rest tilt-test in reducing post-bed rest orthostatic intolerance, as well as their influence on the renal and endocrine responses to orthostatic stress.
- 3) Investigate the effects of different countermeasures in reducing cardiac electrical instability, and to study the effects of these countermeasures on baseline orthostatic tolerance.
- 4) Investigate the effects of different countermeasures in reducing cardiac electrical instability after simulated microgravity, and to study the effects of these countermeasures on orthostatic tolerance and on the renal and endocrine responses to simulated microgravity.

These studies have implications for the treatment and prevention of maladaptive hemodynamic responses experienced by astronauts in flight and on return to Earth. They will provide an innovative way of testing "individualized countermeasures." Finally, the results of these studies may help us better understand the pathophysiology of alterations in volume homeostatic mechanisms in cardiovascular diseases such as congestive heart failure, dysrhythmias and hypertension.

NSBRI RESEARCH PROGRAM HUMAN PERFORMANCE FACTORS

Team Leader: **Czeisler, C. A.** **Harvard**

**Associate Team
Leader:** **Brainard, G. C.** **Thomas Jefferson**

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Gerner, E. W.	CO-I	Thomas Jefferson

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Glickman, G.	CO-I	Thomas Jefferson
Hanifin, J.	CO-I	Thomas Jefferson
Lockley, S. W.	CO-I	REQUEST OTHER
Pineda, C.	CO-I	Thomas Jefferson
Smith, P.	CO-I	Arizona

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Wright, K. P.	CO-I	Harvard
Kronauer, R. E.	CO-I	Harvard
Ronda, J.	CO-I	Harvard

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Rogers, N. L.	CO-I	Penn
Van Dongen, H. P.	CO-I	Penn

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Reid, K. J.	CO-I	Northwestern		

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	George C. Brainard, Ph.D.
ORGANIZATION:	Jefferson Medical College of Thomas Jefferson University
PROJECT TITLE:	Optimizing Light Spectrum for Long-Duration Space Flight
END DATE:	08/31/2004

Project Executive Summary

Risk factors for the health and safety of astronauts and NASA ground control workers include disturbed circadian rhythms and altered sleep-wake patterns. These physiological changes can result in decrements in alertness, concentration, and performance, all of which threaten the safety of personnel and the objectives of space missions. In studies of astronauts and NASA ground control workers, light treatment has been used as an effective countermeasure to provide entrainment of sleep-wake patterns and other circadian rhythms. It is important to optimize light as a countermeasure for circadian and sleep disruption in space flight missions. For civilians, light treatment is being tested for improving circadian entrainment and enhancing both performance and alertness in shift workers. A Congressional report estimates that 20 million full-time workers in the United States are shift workers and that they have increased health problems including higher risk of cardiovascular disease, gastrointestinal distress, as well as cognitive and emotional problems. The long-term goal of our research is to determine the best wavelengths of light for use as a countermeasure during long duration space flight, as well as for adjusting circadian and sleep disruption in civilians.

Towards achieving this aim, an eight wavelength action spectrum (the relative effectiveness of different wavelengths for eliciting a biological response) has been established to help identify the photoreceptor system for light regulation of melatonin in humans (Brainard et al., 2001b). Ultimately, this action spectrum may be used as a tool for investigating the action spectrum and related photoreceptor system involved in circadian entrainment and phase-shifting. One specific aim of the current research is to extend the action spectrum for light-induced plasma melatonin suppression using monochromatic wavelengths below 440 nm and above 600 nm. These wavelengths are relevant to astronauts who have to adapt to extraterrestrial environments that have spectral characteristics different from those found on Earth. As examples, there is substantially increased short wavelength light below 440 nm outside of the Earth's atmosphere, and Martian skylight has an abundance of long wavelengths above 600 nm. Data from the proposed studies can be used to optimize the lighting environment of astronauts on long term missions. Specifically, these data can be used to 1) improve light treatment as a countermeasure for circadian and sleep-wake disruption in NASA space flight missions, 2) identify the best spectral transmission for space suit visors and the windows used in space vehicles and habitats, and 3) engineer the ideal spectral distribution for illumination of general living quarters during space exploration.

Specific Aims:

1. Test the hypothesis that wavelengths below 440 nm and above 600 nm are active in regulating melatonin secretion;

2. Test the hypothesis that there will be a loss of sensitivity to monochromatic light when the eyes are not pharmacologically dilated during the melatonin suppression trials, and;
3. Test the hypothesis that there will be a shift in spectral sensitivity of light regulation of melatonin when the eyes are not pharmacologically dilated.

Key Findings

For Specific Aim One, a key finding is that the fluence-response relationship between nocturnal exposure to 420 nm monochromatic light and melatonin suppression is univariant with the dose response with exposures to wavelengths between 440 and 600 nm. Two recent action spectra suggested that a novel vitamin A₁ retinaldehyde-based photopigment may be primarily responsible for melatonin suppression in humans (Brainard et al., 2001b; Thapan et al., 2001). There was poor agreement between these action spectra, however, on the relative sensitivity to monochromatic light at 420-424 nm, allowing for the possibility that the action spectra could be matched to a cryptochrome absorption spectrum. These 420 nm dose-response data suggest that the melatonin action spectrum of Brainard et al. (2001b) fit an absorption spectrum for a novel opsin photopigment which mediates photoreception for the human retinohypothalamic tract. This finding has practical importance to astronauts in long duration space flight since 420 nm irradiance is greatly increased outside the earth's atmosphere (e.g. on the Space Shuttle and International Space Station).

For Specific Aim Two, a key finding is that it takes up to 56 percent more light at 460 nm light for melatonin regulation when the pupils are free to respond to light stimuli. It will be important to further characterize other wavelength responses in freely constricting eyes in order to practically utilize action spectrum data in optimizing light as a countermeasure to circadian disruption during long duration space flight. In almost all cases, astronauts' eyes will be freely reactive during long duration space flight. The wavelength responses in subjects with freely reactive pupils is currently being pursued in Specific Aim Three.

Finally, additional progress that is relevant to all three specific aims involves the completed design and construction of a new Light Emitting Workstation with three times the photon output of earlier generation Light Emitting Workstations. This high output workstation is particularly important to the ongoing work with long wavelength light above 600 nm. Now operational, the light output of this equipment is so powerful that it requires a separate laboratory sequestered from the lower power workstations. The university has provided the needed laboratory space for this equipment along with the necessary waterlines and drains for cooling and a 220 volt electrical supply for power.

Proposed Research Plan for the Coming Year

1. Complete manuscript including the final 420 nm fluence-response curve.
2. Complete manuscript including two pupillary fluence-response curves.
3. Complete study on wavelength response in volunteers with freely reactive pupils.
4. Complete 630 nm fluence-response curve.
5. Determine feasibility of further work above 630 nm.
6. Test fluence-response sensitivity below 420 nm.
7. Write final report for first three years of this project.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	George C. Brainard, Ph.D.
ORGANIZATION:	Thomas Jefferson University
PROJECT TITLE:	Optimizing Light Spectrum for Long-Duration Space Flight

Project Executive Summary

Risk factors for the health and safety of astronauts and NASA ground control workers include sleep loss and disturbed circadian rhythms. These physiological changes can result in decrements of alertness, concentration and performance, all of which threaten the safety of personnel and the objectives of missions into space. Although studies of astronauts and NASA ground control workers show that light treatment can be used as an effective pre-launch countermeasure to provide entrainment of circadian rhythms and sleep-wake cycles, it has not been used onboard the Space Shuttle or International Space Station. For civilians, light treatment is being tested for improving circadian entrainment and enhancing both performance and alertness in shift workers. A congressional report verifies that shift workers have increased health problems, including higher risk of cardiovascular disease, gastrointestinal distress, as well as cognitive and emotional problems. The long-term goal of the proposed research is to determine the best spectra of light for use as a countermeasure during long-duration space flight, as well as for adjusting circadian and sleep disruption in civilians.

To achieve this aim, it will be determined if polychromatic fluorescent light can be improved as a circadian stimulus by increasing the power in the short wavelength (blue) portion of the spectrum. If broad spectrum light can be enhanced in this way, then onboard artificial lighting systems may serve the dual purpose of maintaining circadian entrainment while providing illumination that supports vision. The proposed aims also include the first test of human neuroendocrine and circadian sensitivity to the ambient light spectra that will be encountered during a long-duration mission on Mars. This will be an important step towards determining if ambient daylight on Mars can be used as a countermeasure for the health and safety of astronauts. Together, these data may be used to 1) improve light treatment as a countermeasure for circadian and sleep-wake disruption in NASA missions, 2) identify the best spectral transmission for space suit visors and windows used in space vehicles and habitats, and 3) engineer the ideal spectral distribution of artificial illumination for astronauts and mission control workers during long-duration space exploration.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Charles A. Czeisler, Ph.D., M.D.
ORGANIZATION:	Harvard – Brigham and Women's Hospital
PROJECT TITLE:	Circadian Entrainment, Sleep-Wake Regulation and Performance During Space Flight
END DATE:	02/29/2004

Project Executive Summary

Optimal human performance during space flight requires astronauts to maintain synchrony between the circadian pacemaker, which regulates the timing of sleep, endocrine function, alertness and performance, and the timing of the imposed sleep-wake schedule. Operational demands of space flight necessitate that humans live on day lengths different than the 24-h solar day of Earth (Dijk et al., 2001). Due to orbital mechanics, astronauts are commonly scheduled to the near equivalent of a shorter-than-24-hour day length in Earth orbit on space shuttle missions; moreover, they will be scheduled to the 24.65-h solar day of Mars on the planned exploration class mission to Mars.

Over the past ten years, we have successfully implemented a new technology for shuttle crewmembers involving bright light exposure during the pre-launch period to facilitate adaptation of the circadian timing system to the inversions of the sleep-wake schedule often required during dual shift missions (Czeisler et al. 1991). However for long duration space station missions it will be necessary to develop effective and attainable countermeasures that can be used chronically to optimize circadian entrainment during extended duration missions.

The purpose of this 65-day long between subjects randomized study is to test three specific hypotheses aimed at evaluating entrainment of the human circadian pacemaker to longer-than-24-hour days.

Specific aim 1: To test the hypothesis that synchronization of the human circadian pacemaker to a sleep-wake and light-dark schedule with an imposed period ~ 4 percent longer than the pacemakers intrinsic circadian period will be disturbed in men and women;

Specific aim 2: To test the hypothesis that this disturbed circadian synchronization will result in the secretion of the sleep-promoting hormone melatonin during the waking day, disturbed sleep, reduced growth hormone and cortisol secretion, and impaired performance and daytime alertness;

Specific aim 3: To test the hypothesis that two relatively brief (45 minutes) daily exposures to evening bright light (~10,000 lux) will establish a normal entrained circadian phase, in subjects whose imposed sleep-wake and light-dark schedule is ~ 4 percent longer than their intrinsic circadian period, resulting in improved sleep consolidation, undiminished endogenous growth hormone and cortisol secretion and enhanced daytime alertness and performance as compared to subjects on the same schedule with out the evening bright light exposure.

These hypotheses are based on the results of our preliminary data which indicate that: (a) the period of the human circadian pacemaker after release from entrainment to the 24-hour day is near to but on average slightly longer than-24-hours (Czeisler et al. 1999), (b) the 24.6-h day is outside the range of entrainment of the human circadian pacemaker in the presence of a weak

environmental synchronizer (Wright et al., 2001), and (c) intermittent exposure to bright light is a cost effective means of resetting the human circadian pacemaker with respect to power use and astronaut time compared to continuous exposure to light (Rimmer et al., 1999).

During FY02 we completed five experiments. This effort amounts to 325 subject test days in the laboratory during year 2, in addition to the 325 subject days that we conducted in year 1. Originally we proposed to complete 260 subject test days per year and are thus 130 subject test days ahead of schedule. Data collected include: Core body temperature, blood samples (melatonin), Urine samples, Sleep and waking EEG recordings, Subjective sleep quality, Actigraphy, Light intensity, neurobehavioral performance and mood. The successful collection of these data will allow us to test hypotheses 1, 2, and 3 of the project. Data analyses are currently in progress.

The plans for the near future are to continue testing subjects on the Earth and Mars day, analyze data collected, and to test as a countermeasure the ability of brief pulses of bright light to synchronize humans to a dim light-dark cycle for the Earth and Mars day lengths.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Charles A. Czeisler, Ph.D., M.D.
ORGANIZATION:	Harvard – Brigham and Women's Hospital
PROJECT TITLE:	Circadian Entrainment, Sleep-Wake Regulation and Neurobehavioral Performance During Extended- Duration Space Flight

Project Executive Summary

NASA's planned exploration class mission to Mars will require crewmembers to adapt to a 24.65-hour solar day throughout their >540-day stay on Mars. The ability of astronauts to sustain a high level of performance during their mission will be critically dependent upon adaptation of their circadian pacemaker to the 24.65-hour day. Our data suggest that most astronauts would exhibit circadian misalignment if the space flight lighting conditions of <25 lux on the windowless middeck of the space shuttle were present during their stay on Mars. Circadian misalignment would result in sleep impairment, endocrine disturbances and impaired neurobehavioral function.

Preliminary data from our laboratory reveal that intermittent bright light exposure is effective in maintaining entrainment of the circadian pacemaker to longer-than-24 hour days. They also suggest that lighting levels of ~25 lux or ~100 lux are insufficient to maintain a normal phase angle of entrainment. Other preliminary results demonstrate that blue light (460 nm) is more efficient than white light in resetting the human circadian pacemaker. Given that bright light pulses might not of practical use on Mars – due to time and energy constraints – these results demonstrate the need to develop practical and cost-effective countermeasures to adapt the human circadian pacemaker of all crewmembers to the 24.65-hour day.

We propose to test a countermeasure of “blue-enriched” light to entrain the human circadian pacemaker to a 24.65-hour day. Specifically, we propose to test the following hypotheses: 1) that synchronization of the human circadian pacemaker to a 24.65-hour day initiated at an adverse phase will not be appropriate in the presence of ~100 lux of white light; 2) that inappropriate circadian synchronization will result in the secretion of the sleep-promoting hormone melatonin during waketime, abnormal somatotropic and corticotropic activity, disturbed sleep, and impaired performance and daytime alertness; and 3) that exposure to “blue-enriched” light during the daytime will establish a normal entrained circadian phase in subjects scheduled to a 24.65-hour day. We have proposed a randomized 71-day clinical trial to test these three hypotheses. The results of the proposed studies will answer fundamental questions on the mechanisms underlying circadian entrainment in humans and could have a profound effect on the health, productivity and safety of astronauts during an exploration class mission to Mars.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	David F. Dinges, Ph.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Countermeasures to Neurobehavioral Deficits From Partial Sleep Loss
END DATE:	06/30/2004

Project Executive Summary

Our research identifies methods to prevent neurobehavioral and physical deterioration due to inadequate sleep and sleep placed at different times across the 24-hour day in astronauts during long-duration manned space flight. The performance capability of astronauts during extended-duration space flight depends heavily on achieving recovery through adequate sleep. Even with appropriate circadian alignment, sleep loss can erode fundamental elements of human performance capability including vigilance, cognitive speed and accuracy, working memory, reaction time, and physiological alertness. When attempting to sleep and perform at an adverse circadian phase, the magnitude and time course of sleep loss and consequent deficits in neurobehavioral functioning are significantly affected. Adequate sleep is essential during manned space flight not only to ensure high levels of safe and effective human performance, but also as a basic regulatory biology critical to healthy human functioning.

There is extensive objective evidence that astronaut sleep is restricted in space flight to averages between 4 hr and 6.5 hr/day. Chronic sleep restriction during manned space flight can occur in response to endogenous disturbances of sleep (motion sickness, stress, circadian rhythms), environmental disruptions of sleep (noise, temperature, light), and curtailment of sleep due to the work demands and other activities that accompany extended space flight operations. The mechanism through which this risk emerges is the development of cumulative homeostatic pressure for sleep across consecutive days of inadequate sleep. Research has shown that the physiological sleepiness and performance deficits can progressively worsen (i.e., accumulate) over consecutive days of sleep restriction, and that sleep limited to levels commonly experienced by astronauts (i.e., 4 - 6hr per night) for as little as 1 week, can result in increased lapses of attention, degradation of response times, deficits in complex problem solving, reduced learning, mood disturbance, disruption of essential neuroendocrine, metabolic, and neuroimmune responses, and in some vulnerable persons, the emergence of uncontrolled sleep attacks.

The prevention of cumulative performance deficits and neuroendocrine disruption from sleep restriction during extended duration space flight involves finding the most effective ways to obtain sleep in order to maintain the high-level cognitive and physical performance functions required for manned space flight. There is currently a critical deficiency in knowledge of the effects of how variations in sleep duration and timing relate to the most efficient return of performance per unit time invested in sleep during long-duration missions, and how the nature of sleep physiology (i.e., sleep stages, sleep electroencephalographic [EEG] power spectral analyses) changes as a function of sleep

restriction, the timing of sleep, and performance degradation. The primary aim of this project is to meet these critical deficiencies through utilization of a response surface experimental paradigm.

Through testing in a dose-response manner, varying combinations of sleep duration and timing, this project will help establish how to most effectively limit the cumulative adverse effects of chronic sleep restriction in space operations on human performance and physiology. Although there is evidence that the less sleep obtained, the greater the waking deficits, experiments have found that for acute periods supplementing a reduced anchor sleep period with a nap, there exists a potential to enhance performance due to the exponential recovery of neurobehavioral functions relative to sleep duration.

During the past 5 years we have been using a response surface experimental approach to systematically determine the chronic (10-day) effects of 18 sleep schedule conditions. There are two experiments in this project. The first experiment involved restricted nocturnal anchor sleep alone and in combination with varying durations of restricted daytime naps on performance, mood, sleep, circadian physiology and hormones. The resulting preliminary response surface maps (RSMs) derived from this dose-response experiment indicate that total sleep time per 24hr is a prime determinant of cumulative neurobehavioral deficits, and that combining a restricted nocturnal anchor sleep with a midday nap can attenuate cumulative deterioration in performance.

In order to complete our understanding of how to optimize performance in the face of restricted sleep in space flight, in the second experiment we have reversed the circadian placement of these 18 anchor sleep + nap sleep conditions (i.e., daytime anchor sleep alone and in combination with varying durations of restricted nocturnal naps. To develop the response surface models, both experiments will require $n=90$ (total $N=180$) healthy men and women to undergo a 14-day ground-based laboratory protocol involving random assignment to one of 18 sleep-ration cells. The 18 sleep ration cells utilized in experiment 1 (nocturnal anchor sleep) will be repeated in experiment 2 (diurnal anchor sleep) for a total of 36 sleep ration cells. The sleep-ration assignments involve 4 anchor sleep durations (4.2, 5.2, 6.2, 8.2 hr) and 6 nap sleep durations (0.4, 0.8, 1.2, 1.6, 2.0, 2.4 hr) crossed to yield a total of 4 anchor-sleep-only conditions, and 14 anchor + nap sleep conditions, spanning a dynamic range of cumulative sleep debts (i.e., from 0 to 40 hr in a 10-day period).

Subjects undergo a wide range of quasi-continuous neurobehavioral performance tests and continuous physiological monitoring of waking EEG, sleep PSG, behavioral motility, and body temperature while living in the laboratory for 14 consecutive days. The laboratory environment is designed to simulate the low light, tight quarters, and lack of social contact with the outside world that will characterize long-duration space flight.

Earth-Based Applications of Research Project

Sleep loss, in particular chronic sleep loss, is becoming increasingly more common in today's global society. Chronic partial sleep loss without adequate recovery sleep leads to what is referred to as "sleep debt". Weekly restriction of sleep to <6.5 hours (h) per night is common in many segments of society including shift workers, long-haul truck drivers, police personnel, medical workers, transoceanic pilots and astronauts. In addition, recent epidemiological studies

reported that between 15-20% of Americans sleep 6.5 h or less per night. Similar estimates have been reported in other populations in Europe, Asia and Australia.

Sleep restriction is associated with increased risk of errors, traffic accidents, injuries, interpersonal conflicts, stimulant use (licit and illicit), and burnout. These behavioral problems are based in altered brain functions due to chronic sleep restriction. Recent laboratory-based, randomized controlled experiments provide extensive evidence that chronic restriction of sleep to 4h, 5h or 6h per day, for periods from 5 to 14 days, results in cumulative neurobehavioral impairments, irrespective of the circadian phase at which the sleep is obtained. Cognitive performance deficits accumulate across days of partial sleep loss to levels equivalent to those found after 1 to 3 nights of total sleep loss. Sleep dose-dependent cumulative effects were also observed in a recent 7-day, laboratory study of truck drivers. In addition to the behavioral effects, chronic sleep loss also poses significant health risks.

Epidemiological studies have found that short sleep (<6.5h per night) is associated with increased risk of coronary heart disease and an overall increased risk of mortality. Laboratory studies of healthy adults subjected to chronic partial sleep loss have found adverse effects on endocrine functions, metabolic responses and immune inflammatory responses. One potential countermeasure to minimize the risk of these neurobehavioral and physiological alterations is to supplement shortened sleep durations with additional, short sleep periods, or naps. This may be especially beneficial when a consolidated nocturnal sleep period is not possible, and individuals are required to be awake across the night and sleep during the day, for example shift workers, and during adjustment to new time zones following transmeridian travel. It is important to determine what duration of nap will provide the most benefit for alertness and cognitive functions, while still avoiding the detrimental effects of sleep inertia following termination of the sleep period.

Information obtained from the current research project will provide important information on the effects of chronically restricted sleep placed at an adverse circadian phase (i.e. diurnally) - with and without nocturnal naps - on neurobehavioral and physiological functioning including sleep physiology, endocrine measures and thermoregulation. In addition to providing information on the effectiveness and effects of different split sleep-wake schedules for use in spaceflight, this information will also be applicable for a large number of Earth-based industries and individuals who are chronically exposed to sleep restriction. Ultimately this will help improve health and safety of individuals on Earth.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	David F. Dinges, Ph.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Countermeasures to Neurobehavioral Deficits from Cumulative Sleep Deprivation During Space Flight: Dose-Response Effects of Recovery Sleep Opportunities

Project Executive Summary

The optimal performance of astronauts during extended-duration space flight depends heavily on achieving recovery through adequate sleep. This project will develop sleep schedule countermeasures to ensure neurocognitive performance capability in astronauts during prolonged space flight. Sleep is chronically restricted in space to four-six hours per day for reasons often associated with operational requirements. Ground-based studies reveal that such chronic sleep durations result in cumulative performance impairments.

The experiment will establish the countermeasure benefits for performance from an acute increase in recovery sleep duration that occurs between two periods of chronic sleep restriction. In addition, generating sleep dose-response functions will provide needed information on the adverse performance consequences of an acute reduction in sleep duration, which can occur in space flight prior to a day of critical operations.

A sleep-duration, dose-response experimental approach with randomization to condition will be carried out on N=80 healthy adults (n=40 females; n=40 males). Sleep duration dose will be varied parametrically on one night (zero hours, two hours, four hours, six hours, eight hours, 10 hours, or 12 hours), placed midway between two six-night periods of chronic sleep restriction (four hours/night). The resulting dose response curves will quantify, for the first time, the degree of recuperation and/or further decrement of neurobehavioral functions relative to varying amounts of sleep following a period of cumulative sleep loss. In addition, we will resolve whether complete neurobehavioral recovery from chronic sleep restriction is possible within two nights of extended sleep duration.

Subjects will be monitored for a wide range of neurobehavioral performance functions, fatigue and mood states, waking EEG, core body temperature, behavioral motility, cardiovascular activity and sleep PSG, while living for 17 days in a laboratory setting that simulates the low light, tight quarters and lack of social contact with the outside world, characterizing long-duration space flight. The results have the potential to fill critical gaps in scientific understanding of the impact of sleep duration on recovery from prior chronic sleep debt; inform and enrich biomathematical models of performance in space flight; and help identify the importance of the strategic use of periodic recovery sleep durations in the many Earth-based occupations in which chronic sleep loss poses a risk to health and safety.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Russell G. Foster, Ph.D.
ORGANIZATION:	Imperial College of Science Technology and Medicine
PROJECT TITLE:	The Role and Characterization of Novel Photoreceptor Mechanisms Regulating Circadian Rhythms, Sleep, Body Temperature and Heart Rate

Project Executive Summary

Varied aspects of physiology and behavior are regulated by gross changes in environmental light. Research leading up to this proposal has shown that in both man and rodents some of these irradiance changes are detected by novel ocular photoreceptors. The photoreceptive mechanisms underlying these, non-rod, non-cone photoreceptors remain uncharacterized. Yet their importance in our daily lives is profound. It is now clear that light exposure can influence alertness and sleep propensity. Furthermore, light regulates the phase of circadian clocks, and thus the timing of rhythmic functions such as digestion, sleep and performance.

The central aims of the research outlined in this application are to characterize the molecular mechanism of this unexplored photoreceptor system of the eye, and determine the extent to which these photoreceptors contribute to varied aspects of physiology and behavior. Employing a unique rodless and coneless mouse model (rd/rd cl), and taking advantage of the new opportunities created by a range of post-genomic technologies, including the imminent completion of sequences for both mouse and human genomes, we will address two questions.

Question One: What is the role of novel photoreceptors in the regulation of general physiology and behavior? The experiments in this section will determine the extent to which body temperature, heart rate (ECG) and EEG are modulated by non-rod, non-cone ocular photoreceptors. Furthermore, we will define the relationship between novel ocular photoreceptors, light, sleep state and levels of c-fos expression in the ventrolateral preoptic nucleus (VLPO) of the brain.

Question Two: What are the molecular mechanisms of non-rod, non-cone ocular photoreceptors? Three broad strategies will be undertaken based upon bioinformatics, proteomics, and microarray technology. Our aim will be to identify a set of genes that: a) share sequence similarity with proteins known to be involved in photoreceptor/sensory cell function; b) are expressed in light sensitive cells of the inner retina; c) undergo post-translational modification and/or changes in expression following light exposure.

The results from these studies will provide the mechanistic substrate for both targeted drug development aimed at the manipulation of human circadian rhythms, sleep, mood and performance, and the design of new lighting sources that are either highly effective in regulating these novel photoreceptors or leave them largely unstimulated.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Charles A. Fuller, Ph.D.
ORGANIZATION:	University of California, Davis
PROJECT TITLE:	Circadian Rhythms in the Martian Environment

Project Executive Summary

To maintain health and homeostasis, an organism must regulate each of its physiological systems in concert with all of the others and with the external environment. The Circadian Timing System (CTS) has evolved to allow coordination of an organism's physiology and behavior both internally and with the external 24.0 hr terrestrial day. The mammalian CTS is adapted to the lighting environment found on Earth. As we move toward exploration-class space missions, we will be exposing astronauts to non-Earth environments for increasing lengths of time. Changes may include altered gravity and spectral, intensity and day-length differences. This raises the concern of whether or not humans will be able to synchronize to such an alien environment. For example, a Mars-type exploration would entail stays on Mars of one to two years.

Compared with the Earth, the Martian environment has a photic spectrum shifted to the red, low illumination level, a periodicity of 24.62 hr, and a 0.38 G gravitational field. The mammalian CTS is most sensitive to light of the blue-green wavelengths and adapted to synchronize to a 24.0 hr day. In addition, light must be relatively bright to affect the CTS of primates, especially humans. Further, altered CTS function - including rhythm amplitude and wave form, sensitivity to light and CTS period - have been reported in both the microgravity environment of space flight and in hyperdynamic fields on the Earth. This program will examine the ability of primates (male and female rhesus monkeys) CTS to cope with the Martian environment.

The first three experiments will examine responses to the Martian day, while the last experiment will examine the effects of G on the period of the circadian pacemaker. Experiment 1 will examine the ability of the CTS to synchronize to the Martian photic (spectrum and period) environment. We will examine long-term (4-month) physiological and behavioral responses. Experiment 2 will similarly examine long-term responses to a photic environment composed of a Martian day and Earth light spectrum. Experiment 3 will use the primate model to initiate the development of countermeasures to assure optimum entrainment of the CTS. This experiment will examine the effects of timed bright light pulses on CTS entrainment.

Using the forced desynchrony protocol, Experiment 4 will examine the effects of 1.0, 1.5 and 2.0 G on the period of the circadian pacemaker. We will develop a G vs. period model to predict the effect of the 0.38 G Martian environment on the period of the circadian pacemaker. This model will be used to develop countermeasure requirements to be tested in Experiment 3. Thus, this program will develop a primate model to evaluate physiological and behavioral consequences of long-term exposure of male and female subjects to altered lighting and gravitational environments.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Megan E. Jewett, Ph.D.
ORGANIZATION:	Harvard – Brigham and Women’s Hospital
PROJECT TITLE:	Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure
END DATE:	03/31/2004

Project Executive Summary

The Original Aims of the Project:

Specific Aim 1: To further develop and refine our ‘Dynamic Stimulus Processing’ Light Model so that it can accurately predict the phase and amplitude of the human circadian system under any lighting conditions, especially those that occur in space. This will be done using data from four completed studies of the effects on the human circadian system of: *i*) three-cycles of brief bright light pulses; *ii*) three-cycles of extended bright light pulses; *iii*) three-cycles of extended low- and moderate-intensity light pulses; and *iv*) single- and double-cycles of amplitude-suppressing critically-timed extended bright light pulses.

Specific Aim 2: To validate the Light Model refined above in Specific Aim 1 using data from four other completed studies of the effects on the human circadian system of: *v*) single-cycle patterns of brief bright light pulses; *vi*) single-cycle extended bright light pulses; *vii*) single-cycle extended light pulses across a wide range of intensities; and *viii*) sleep-wake/light-dark schedules with a wide range of periods (11-h, 20-h, 23.5-h, 24-h, 24.6-h, 28-h, 42.85-h), different light intensities during wake (1, 8, or 15 lux), and with or without a single exposure to an extended bright light stimulus (in the 11-h condition only).

Specific Aim 3: To incorporate the Light Model refined and validated above in Specific Aims 1 and 2 into our mathematical Neurobehavioral Performance Model, which will then be validated against experimental performance data collected under the wide variety of lighting conditions encompassed in the eight studies described above in Specific Aims 1 and 2.

Specific Aim 4: To develop a user-friendly *Circadian Performance Simulation Software (CPSS)* package that can be used to specify appropriate light schedules as a countermeasure to the poor performance and sleep quality associated with circadian misalignment in space.

The Key Findings of the Project:

1. We refined and validated our current circadian pacemaker model to predict the effects of different stimuli on entrainment and phase resetting of pacemaker at low light levels.
2. We characterized the amplitude recovery dynamics of the endogenous pacemaker as well as identified different excitation regions of the pacemaker.
3. Our user-friendly Circadian Performance Simulation Software (CPSS) package was tested with actual mission schedule to evaluate our predictions of performance and alertness during pre and post-launch conditions.

4. A preliminary algorithm for schedule assessment and countermeasure design was developed based on the analysis of different schedules using CPSS.

The Impact of these Findings:

1. The impact of finding 1 above suggests that the circadian pacemaker can be entrained using different stimuli and the current mathematical model can be used for simulating the effect of flight on pacemaker dynamics during long duration space missions.
2. The impact of finding 2 above suggests the rate of recovery from amplitude reduction is slower and therefore returns to equilibrium conditions may take longer. This has implications for the rate of recovery after disruption of the pacemaker (e.g. by different shift schedules or lighting conditions). Amplitude reduction of the pacemaker may affect the influence of circadian pacemaker on sleep consolidation or neurobehavioral performance during the daytime.
3. The impact of finding 3 is that there is now available a software package to evaluate our predictions of the performance and alertness of crewmembers during long-duration space missions
4. The impact of finding 4 is that the algorithm can be incorporated into our software package to assess the schedules of long duration space missions in order to design appropriate countermeasures to improve the performance and alertness of crewmembers on these missions.

Earth-Based Applications of Research Project

This research focuses on the further development of mathematical models and software that aid in schedule design to improve performance (and thereby public safety) for people who work at night, on rotating schedules or on non-24-hour schedules (pilots, train and truck drivers, shift workers, health care workers, etc.). This research also aids in the specification of lighting requirements aboard spacecraft and in other work conditions to insure proper entrainment and circadian phase of these workers, even if they work at night or on rotating schedules.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Elizabeth B. Klerman, M.D., Ph.D.
ORGANIZATION:	Harvard – Brigham and Women’s Hospital
PROJECT TITLE:	Mathematical Modeling of Circadian/Performance Countermeasures

Project Executive Summary

Objective neurobehavioral performance, subjective alertness and mood, and sleep are critically important to astronaut health and the success of space missions. Neurobehavioral performance, alertness and mood are affected by circadian rhythms, homeostatic sleep regulation, sleep inertia, and interactions of these processes. Countermeasures to ensure optimal neurobehavioral performance, subjective alertness and quality sleep therefore are required for space missions, during which circadian rhythms and sleep are disrupted. We have developed and validated a mathematical model of the human circadian pacemaker and neurobehavioral performance and alertness that includes these three key processes.

A previous version of this model, with a focus on light-dark scheduling, has been used by NASA to design astronaut pre-launch schedules. We propose to extend this model to be useful in testing emerging countermeasures for neurobehavioral problems due to space missions. Since the potential countermeasures, singly or in combination, are different for each crewmember on each mission, it would be difficult, time consuming and expensive to conduct all the experimental protocols required to mimic all combinations of possible situations and proposed countermeasures received by any given crewmember. A mathematical model, on the other hand, is a powerful tool for the design of countermeasures because there are no limits to the number of patterns of astronaut light exposure or sleep/wake schedules and countermeasures that can be efficiently assessed. Our model is available for use via a user-friendly software program for users to test countermeasures.

We propose to extend the current model so that it will include: (1) melatonin markers of circadian amplitude and phase; (2) chronic sleep restriction and its effects on neurobehavioral performance; and (3) the effects of specific wavelengths of light on the circadian pacemaker. Then we will amend our current software to include schedule assessment and countermeasure design components. We will cooperate with other members of the selected NSBRI Human Performance Factors Team: simulating their protocols, modeling the data and adjusting and re-validating the model as required. The mathematical modeling of circadian rhythms, sleep, subjective alertness and mood, and neurobehavioral performance is a vital and effective component of design and testing of potential countermeasures for optimal astronaut health and mission success.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Michael Menaker, Ph.D.
ORGANIZATION:	University of Virginia
PROJECT TITLE:	A Model of Circadian Disruption in the Space Environment
END DATE:	06/30/2004

Project Executive Summary

In order to discover countermeasures against the deleterious physiological and behavioral consequences of the inevitable disruption of normal circadian rhythmicity produced by the conditions in space, we have first to create a laboratory model of that condition (which for the sake of brevity we are calling "dysphasia"). To be successful such a model must enable us to measure the effects of simulated space conditions on multiple body functions as well as on the temporal relationships of these functions to each other and to the environment. These conditions are fulfilled in large part by our transgenic rat model in which the transcription of the clock gene *Per1* is reported in real time by luciferase. We are able to culture tissues from such rats and to measure the phase of their circadian rhythms in vitro, enabling us to infer their phase relationships in the intact animal. Our aims are first to determine how these phase relationships are disrupted by simulated space conditions, and second to devise counter measures that could in practice be employed by astronauts in space to reinstate normal temporal organization. Of necessity, to be practical, countermeasures must be compatible with the ongoing activities in space vehicles. It is therefore impractical to use the strongest known synchronizing signal, a regular 24-hour light cycle.

We have investigated an alternative synchronizing signal, precisely-timed meals, and have found that its effects are stronger than anticipated and extend deeply into the physiology of the animal. Timed meals set the phase of the circadian rhythms of gene expression in liver, stomach, colon, esophagus, lung, and also the phase of locomotor behavior. Timed meals do not influence the phase of gene expression in the suprachiasmatic nucleus (SCN) or the femoral artery. Our results suggest that timed meals may prove to be a useful partial countermeasure against dysphasia which could be combined with other signals (e.g., melatonin) that preferentially target SCN and/or the cardiovascular system. During the next year we plan to test these hypotheses on transgenic rats made dysphasic by exposure to bright constant light.

The approach outlined above depends on inferences about the behavior of tissues in intact animals based on their behavior in culture. It will be important to confirm these conclusions by direct measurement of the same rhythms in intact animals. This is a technically demanding undertaking, but we are making slow progress by recording luciferase activity with implanted light guides in awake, behaving animals. We will continue to refine this approach.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Michael Menaker, Ph.D.
ORGANIZATION:	University of Virginia
PROJECT TITLE:	A Model of Circadian Disruption in the Space Environment

Project Executive Summary

Our transgenic rat model enables us to measure circadian rhythms of transcription of a "clock gene" (Per1) in the brain and peripheral tissues. We have shown that Per transcription is circadian in *in vitro* preparations of suprachiasmatic nucleus (SCN), several other brain areas, liver, lung, muscle, tissues of the cardiovascular system and other peripheral organs. Thus each of these areas and organs contain circadian oscillators – those in the SCN persist for more than a month *in vitro*, while those in other areas damp out more or less rapidly. These data support a model of mammalian circadian organization in which the SCN acts as a synchronizer of rhythms in other brain areas and in the periphery. "Abnormal" environments, such as those that will be encountered in space travel, disrupt the normal organization of the mammalian circadian system.

We propose to identify the signals that couple the SCN to peripheral oscillators in preparation for the design of countermeasures that will compensate for this disruption. We anticipate that there may be several (many?) such signals and that some may be redundant. That makes their identification difficult; however it also suggests that it will not be necessary to replace all the natural signals in order to restore circadian order.

The literature and our own preliminary work suggest four likely signals: melatonin, adrenal steroids, sympathetic neural input (epinephrine and norepinephrine) and parasympathetic input (acetylcholine). We will examine the effects of removing (or altering) these potential signaling pathways singly and in combination. Where possible we will use surgical ablation; where that is impractical we will make chemical lesions. Circadian organization will be evaluated in lesioned animals by examining the phases of cultured peripheral tissues in animals exposed to normal light-dark cycles, phase-shifted light-dark cycles, constant light and restricted (timed) meals.

Using information derived from the above experiments, we will design and test countermeasures. We will focus our efforts on those procedures which, if they prove effective in our animal model, can be applied directly to humans since they are either noninvasive (timing of light or meals) or have been shown to be relatively safe (melatonin), and commonly used drugs that affect the autonomic nervous system.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Lawrence P. Morin, Ph.D.
ORGANIZATION:	State University of New York – Stony Brook
PROJECT TITLE:	Circadian and Vestibular System Relationships

Project Executive Summary

The series of studies proposed in this grant, "Circadian and Vestibular System Relationships," is entirely novel. There is no scientific literature in existence concerning the relationship between the two systems in the grant title. However, at the time the proposal was submitted, a paper had been just been published in the *Journal of Comparative Neurology* by Shiroyama and colleagues (1999) showing a direct connection between the medial vestibular nucleus and the ventral lateral geniculate nucleus. However, their data were misinterpreted and the projection is actually to a neighboring nucleus, the intergeniculate leaflet (IGL) of the circadian rhythm system. This opened a lot of possibilities that we developed into a proposal.

Progress with Respect to Specific Aims

Specific Aim One: Connections between the circadian rhythm system and the vestibular system. The first objective has been completed. Using retrograde tracer applied to the IGL, we have shown neurons in the vestibular system project to a nucleus (the IGL) of the circadian rhythm system. We have completed the two planned variations on that anatomical theme. One employed anterograde tracer applied to the medial vestibular nucleus to trace projections to the IGL. The other has utilized a technique novel to this laboratory and has required significant modifications in standard operating procedure. This method uses a transynaptic viral tracer to determine whether two neurons are connected. We have obtained two results of significance: (1) Use of the virus tracing method by itself demonstrates the existence of cells in the vestibular nuclei that have polysynaptic input to the SCN. (2) Use of the virus tracing method in conjunction with a monosynaptic tracer injected into the IGL and the MVe has demonstrated that at least a few of the medial vestibular neurons with multisynaptic projections to the SCN are among those cells that project to the IGL. Furthermore, MVe project to multiple sites which provide afferent input to the IGL, including those which mediate arousal and REM sleep. The results confirm our original expectation that neurons in the medial vestibular nucleus connect to neurons in the IGL that, in turn, project to the circadian clock in the suprachiasmatic nucleus (SCN).

Specific Aim Two: Functional activation of the vestibular and circadian systems by an OKN stimulus. We are assembling the full apparatus and the experiments in this aim are about to start.

Specific Aim Three: Functional activation of the vestibular and circadian systems by a non-locomotor, non-photic stimulus. Functional implications of a vestibular system activating stimulus are being completed. Thus far 86 animals have been subjected to linear acceleration/deceleration and rotational stimulation. After the stimulation, the brains were removed, fixed and processed to determine the extent and location of induction of the immediate early gene, *fos*, as indicated by the presence of FOS protein. Preliminarily, the results show a linear relationship between rate of rotation and number of cells in the IGL (among other places) expressing FOS in a lateralized vestibular-stimulus specific manner. Many more animals have been added to the experiment. The data from those brains and target nuclei are in the process of being entered into the computer for analysis in the near future, based on results which emerged from the extended

anatomical study (Specific Aim 1). The ability of the identical vestibular activating stimulus to elicit circadian rhythm phase shifts is being evaluated. The preliminary data indicate that no phase shifts occur; however, there are clear effects on level of post-stimulus motor activity which are differentiated based on the magnitude of vestibular stimulation.

Impact of the Results on the Specific Aims

There are two significant points of impact. *One* concerns the original set of specific aims. Were they realistic and worthy of experimental study? The answer is clearly affirmative. There are no negative changes in the specific aims. We have added rotational stimulation to the originally proposed linear acceleration/deceleration as part of work done for Specific Aim 3. *The second* point of impact concerns the significance of the system being studied with respect to health risks during space flight. The vestibular system influences has a generally pervasive influence on normal behavior. Therefore, knowledge about the routes and mechanisms through which this influence is achieved may be important. In particular, sleep and circadian rhythms may be profoundly disturbed by high level vestibular activation, or might be actually facilitated by low level vestibular activation.

Research Plan for the Next Year

We expect to continue with the approach thus far. With respect to the anatomical work, we are in the final stages of completing a major paper for submission. The text is >95 percent complete and we are selecting, editing and finalizing the figures and verifying all the statements of anatomical "truth." We will finish analysis of FOS protein induction in elements of circadian pathways by vestibular activation. We will complete the study of vestibular activation and circadian rhythm phase shift. We will complete studies of circadian rhythm phase shifts by optokinetic stimulation.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Gianluca Tosini, Ph.D.
ORGANIZATION:	Morehouse School of Medicine
PROJECT TITLE:	Long-Term Exposure to Dim Lighting Desynchronizes the Circadian System of Rats
END DATE:	08/31/2004

Project Executive Summary

Many biochemical, physiological and behavioral parameters exhibited by organisms show daily fluctuations and most of these daily rhythms persist in constant conditions, thus, demonstrating that they are driven by endogenous oscillators. The rhythms that persist in constant conditions with a period close to 24 hours are called circadian rhythms. One the most important aspects of space flight is the absence of geophysical 24-h cycles, which, of course, affects the overall temporal organization of the organisms. In the case of long-duration manned space flight, it is crucial to understand how the whole circadian system would react and behave in such circumstances.

We discovered that exposing rats to constant dim light for 60 days significantly affected the phase-relationship among circadian outputs in the SCN, retina and pineal, demonstrating that in these animals internal desynchronization of the circadian rhythms is occurring. We also observed that the circadian rhythm in arylalkylamine N-acetyltransferase (the enzyme that is responsible for the circadian rhythm in melatonin synthesis) was altered in both the retina and in the pineal gland. Our data also indicated that locomotor activity rhythm might be an unsatisfactory marker to assess the circadian status of the whole organism. Internal desynchronization has profound effects on the capability of the organisms to perform (mentally and physically) and to remain healthy. In this research proposal, we have designed a series of experiment aimed to understand the mechanisms that are responsible for the observed desynchronization. We believe that the model we have generated will be useful in to foreseeing and preventing dysfunction of the circadian system that may arise after long periods in the space environment where the normal cycle has been altered.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Gianluca Tosini, Ph.D.
ORGANIZATION:	Morehouse School of Medicine
PROJECT TITLE:	Preventing Desynchronization of the Circadian System in Long-Term Space Flight

Project Executive Summary

Many biochemical, physiological and behavioral parameters exhibited by organisms show daily fluctuations and most of these daily rhythms persist in constant conditions, thus, demonstrating that they are driven by endogenous oscillators. The rhythms that persist in constant conditions with a period close to 24 hours are called circadian rhythms. One the most important aspects of space flight is the absence of geophysical 24-hour cycles, which, of course, affects the overall temporal organization of the organisms. In the case of long-duration manned space flight, it is crucial to understand how the whole circadian system would react and behave in such circumstances.

We discovered that exposing rats to constant dim light for 60 days induces internal desynchronization in 20-30 percent of the rats tested. In this project, we plan to further investigate this phenomenon, and to test specific countermeasures to obviate the occurrence of internal desynchronization in animals exposed to constant dim light. In particular, we will test if melatonin injection and exposure to brief pulses (skeleton photoperiod) of light (white light and light of different wavelengths) can prevent internal desynchronization. Internal desynchronization has profound effects on the capability of the organisms to perform (mentally and physically) and to remain healthy. In this project, we have designed a series of experiments aimed to understand the mechanisms that are responsible for the observed desynchronization and to test specific countermeasures aimed to prevent internal desynchronization. We believe that the model we have generated will be useful in foreseeing and preventing dysfunction of the circadian system that may arise after long periods in the space environment where the normal cycle has been altered.

RESEARCH AREA:	Human Performance Factors, Sleep and Chronobiology
PRINCIPAL INVESTIGATOR:	Fred W. Turek, Ph.D.
ORGANIZATION:	Northwestern University
PROJECT TITLE:	Animal Model for Sleep Loss and Circadian Disruption
END DATE:	07/31/2004

Project Executive Summary

The adverse effects associated with imposed disruptions of the normal circadian and sleep-wake cycles are particularly relevant to NASA personnel and their ability to carry out normal duties at a high level of efficiency. Many space travel situations demand that both ground-based and flight personnel engage in duty schedules that can lead to circadian rhythm disruption and sleep loss. The tasks that can be affected involve vigilance, operation and control of vehicles/aircraft, maintenance, preparation and operation of equipment as well as command and control activities. Night operations are important for successful missions, and there is a clear need to find countermeasures that can alleviate the adverse effects of these activities on human circadian rhythms and sleep as well as on neurobehavioral capabilities and on physical performance.

Despite the high prevalence of chronic partial sleep loss and circadian disruption due to shiftwork in modern society, no animal models have previously been developed to systematically examine the effects of chronic partial sleep and circadian disruption on sleep architecture and performance. The use of a new animal model, as outlined in the original proposal, will lead to new insights into how the circadian and sleep systems are affected by the disruption of their normal phase relationship to one another, and how this temporal disorganization influences neurobehavioral capabilities and motor performance. Information gained using this novel animal model will also be important in the development of effective countermeasures to the adverse effects associated with circadian disruption and sleep loss. These countermeasures could be useful in a number of situations involving NASA personnel, particularly in extended duration space flight missions that will result in challenges to the sleep and circadian system of the flight crew and support teams. This project will also provide important insights in to the interactions between the circadian and sleep/wake systems.

Specific Aims

There are three specific aims of the project: 1) to determine the effects of 12 hours of imposed wakefulness during both normal active and inactive periods on circadian rhythms, the sleep-wake cycle and neurobehavioral and motor performance measurements, 2) to test the hypothesis that treatment with either a physiological or pharmacological dose of melatonin at the beginning of the imposed period of wakefulness will alter the effects of this temporal desynchrony on the circadian clock, the sleep-wake cycle, and/or on neurobehavioral and motor performance measurements and, 3) to test the hypothesis that access to a wheel (exercise) when in the home cage will alter the effects of the imposed periods of wakefulness on the circadian clock, the sleep-wake cycle, and/or neurobehavioral and motor performance measurements.

During the award period we have examined the impact of chronic partial sleep loss and circadian disruption on sleep, circadian rhythms and neurobehavioral and motor performance. With the development of an animal model of sleep loss and circadian disruption, we have determined that mice respond in a similar way to chronic partial sleep loss and circadian disruption as humans.

Sleep is altered depending on the strain and the time-of-day of sleep restriction. During our forced wakefulness procedure animals are not able to get any REM sleep but can get anywhere between 5 to 40 % NREM sleep. Over the 10 day period of partial sleep restriction animals are sleep deprived of between 26 to 41 hours of sleep, depending on the strain and time of sleep restriction (i.e. light or dark period). The degree of sleep loss seen in these studies is equivalent to a human obtaining approximately 5-6 hours of sleep per night, which is commonly seen on shuttle missions. We have also determined that this moderate degree of sleep deprivation does not significantly impair performance on a task of neurobehavioral and motor performance. The data collected during this unique study appears to be similar to data recently published on human subjects exposed to chronic partial sleep loss.

**NSBRI RESEARCH PROGRAM
IMMUNOLOGY, INFECTION AND HEMATOLOGY**

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RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	Janet S. Butel, Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Viral Infections and Mucosal Immunity
END DATE:	03/31/2004

Project Executive Summary

Space flight has been found to affect immune responses, and alterations in a normal immune response could have a major impact on the host's ability to control infections. An important question being explored is whether infectious diseases will pose an unacceptable medical risk to the success of long-duration space journeys. All humans are infected for life with latent and persistent viruses, and it is well-known that suppression of the immune system allows latent viruses to reactivate and multiply, which may cause disease in the person undergoing reactivation or in contacts to whom the virus is transmitted.

The general hypothesis being addressed is that conditions of long-duration space flight will alter human immune responses, leading to reactivation of latent viruses, increased viral infections and viral disease, and possible development of malignancies, and to altered mucosal immunity, an important host defense against microbial infections. We are focusing on reactivation and shedding of human herpesvirus EBV and human polyomaviruses, agents known to establish persistent infections and to undergo reactivation and cause disease, including cancer, when the host immune system is compromised. Animal models are being used to study radiation effects on host responses to infections.

The original aims of the project were:

1. To determine the effect of space flight conditions on virus reactivation, infection and replication using ground-based models that simulate aspects of space flight;
2. To determine the effect of irradiation on the immune system, susceptibility to virus infection, and development of virus-induced cancers in murine animal models; and
3. To characterize global changes in mucosal immune responses under simulated space flight conditions in the hind limb unloading (suspended mouse) model.

Key findings of the project this year were from the mouse polyoma virus space radiation model we developed. Preliminary studies suggest that combined effects of radiation and virus infection on the immune system of experimental animals lead to immunosuppression and latent virus reactivations. Because of the observed effects upon immunocompetence and viral replication, it is likely that future studies will be able to offer surrogate marker tests for virus infection and immunocompromise, and new approaches to the prevention and treatment of latent virus reactivation as well as lymphoid malignancies that develop as a consequence.

Earth-Based Applications of Research Project

Space flight-induced alterations in the immune system, if serious enough, would have marked adverse effects on host control of microbial infections. Consequences of altered host immunity could result in virus reactivation, replication and disease development, including cancer. As all humans are infected for life with latent or persistent viruses, those infections will be uninvited travelers on all space missions and it is prudent to understand the implications of their presence. Information from our study will guide decision-making regarding countermeasure development, including vaccines to boost antiviral immunity, the use of immune modulators to stimulate components of the immune system to keep viral infections in check, and antiviral

pharmaceuticals to treat reactivated viruses. Such data will be applicable to earth-bound individuals at risk of suffering similar virus reactivations and serious, sometimes life-threatening, consequences due to immunosuppression following organ transplantation or cancer chemotherapy and during pregnancy, old age, and AIDS.

RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	Janet S. Butel, Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Biology of Virus Infections: Radiation and Immunity

Project Executive Summary

The general hypothesis being addressed in this project is that conditions of space flight, including solar radiation, will damage the human immune system, leading to reactivation of latent viruses, increased viral infections and disease, and possible development of cancer. We will continue to focus on human herpesviruses and polyomaviruses; we established in the previous funding period that these viruses, known to cause human disease, are reactivated and undergo increased replication in humans under space flight conditions. We will extend our studies to address the synergistic effects of space radiation and hind limb unloading on viral pathogenesis and host control of infections. Mouse models we have developed involving murine polyoma virus will be used to delineate mechanisms underlying space flight-induced immune changes and infectious disease processes and to test countermeasures.

The specific aims of the proposed study are:

- (1) Determine the effects of space radiation and hind limb unloading on host control of virus infections and virus-induced cancers and develop countermeasures to minimize adverse effects of virus infections. The model system is polyoma virus infections in mice. Gamma radiation effects on virus clearance, virus reactivation, and virus-induced tumor development will be determined. Antiviral drugs and a potential virus vaccine will be tested as candidate countermeasures.
- (2) Characterize direct effects of radiation on viruses and virus-infected cells. Radiation-induced changes in expression of herpesvirus EBV latent and lytic genes will be determined using cell-based assays in vitro, as well as effects of polyomavirus regulatory region variations on viral responses to radiation.
- (3) Collaboration to develop a virus detection and quantification method for inflight monitoring. A molecular beacon-based assay targeted to herpesviruses will be developed.

The project will provide new insights into the effects of space radiation and space flight conditions on infectious disease processes and will help develop facile, spacecraft-compatible methods for detection and monitoring of virus reactivation.

RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	George E. Fox, Ph.D.
ORGANIZATION:	University of Houston
PROJECT TITLE:	Microorganisms in the Spacecraft Environment
END DATE:	03/31/2004

Project Executive Summary

Our work in the past year was focused on four major project areas. We have completed development of a set of 16S rRNA targeted probes that will indicate the presence of major problem and indicator bacteria in flight samples. These probes can be used with similar efficiency under a standard set of operating conditions. The utility of these probes was demonstrated on several samples isolated from various systems including Mars and Moon soil simulants. We also completed the development of a computational algorithm that allows identification of short 16S rRNA subsequences that are highly characteristic of various phylogenetic groupings. In principle, an array of appropriately designed probes based on these signature sequences could be used to determine the genetic affinity (nearest known relatives) in the absence of any prior knowledge of what the problematic organism might be. In practice, the signature sequences themselves tend to be short (15 nucleotides or less) and hence not ideal for use in arrays. We are currently developing an extension to the original algorithm that will allow us to identify very long (30-60 nucleotides) signature fragments whose sequences, despite occasional mismatches, are highly characteristic of various phylogenetic groupings.

An effective set of probes may have utility in a large variety of formats. It is likely that actual implementation in space flight will be driven by mission instrumentation capabilities. Although there is considerable interest in development of array-based instruments other approaches may be preferable, especially for International Space Station applications. We therefore have focused attention on several alternative formats as well. In this regard, several probes for organisms of primary interest have been successfully implemented in a molecular beacon format. Homogenous solution assays of this type would require minimal sample processing and could be readily conducted by astronauts in flight with results signaled by the presence or absence of color changes. It was found that "red-shifted" beacons have minimal contributions from sample autofluorescence. In addition, we demonstrated the potential utility of fluorescent nucleotides such as 2-aminopurine in molecular beacon applications. Efforts were also initiated late in Year 2 to assess the possibility of identifying signature oligonucleotides with mass spectrometry.

Regardless of the assay system ultimately chosen, rapid and simplified systems for sample processing in space will be required. During the past year, we provided further evidence that RNA/DNA purification using compaction agents eliminates the need for preprocessing steps and that the same agents can be used to enhance the adsorption capacity of anion exchangers. Also in the past year we developed further evidence that immobilized metal affinity chromatography (IMAC) which is widely used with proteins is also effective with nucleic acids. Most recently, we have begun to look at novel ways of obtaining rapid final purification of specific RNAs such as 16S rRNA.

There is preliminary evidence that the microgravity environment seen in space effects bacteria in non-obvious ways with such possible outcomes as altered drug resistance or pathogenicity. In order to explore this possibility further, we are examining the response of *E. coli* cells grown in simulated microgravity. In order to do this, we are using modern proteomics technology to examine the expression levels of each and every gene in *E. coli* when cells are grown in a low

shear modeled microgravity (LSMGG) environment. Various kinetic controls have been completed and initial hybridizations with organisms grown under simulated microgravity have been made. These initial studies point to several interesting clusters of genes that appear to be part of a specific response to the LSMMG. In the coming year, we will complete replicates of these initial experiments and examine the results in a multiorganism context.

Overall, Year 2 of the project was very productive. During the past year, four-peer review papers were published, three more are in press, and five additional papers have been submitted. In addition, a book chapter is in press.

RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	Alan M. Gewirtz, M.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Effect of Deep-Space Radiation on Human Hematopoietic Stem Cells
END DATE:	07/31/2004

Project Executive Summary

Astronauts on long-term missions in deep space will be placed at risk from a variety of hazards. Some of these are known while others may be anticipated. Damage to hematopoietic stem cells as a result of radiation exposure is as an example of the latter. Our long-term goal is to identify and quantitate the risks of deep-space radiation to the human hematopoietic system, with particular emphasis on the hematopoietic stem cell.

Stem cells are the ultimate source of both the blood and immune systems, and damage to these cells could have grave immediate and long-term consequences. At the same time, because these cells can be readily removed from the body, manipulated and stored, they are also unique candidates for countermeasures that might obviate, or totally negate, damage incurred to them. Accordingly, this project will have three specific aims which support our long-term goal and these are to:

1. Investigate the cellular consequences of exposing human hematopoietic stem (HSC) and progenitor (HPC) cells to an environment which simulates the radiation environment of deep space;
2. Examine the molecular consequences of exposing human hematopoietic stem cells to an environment which simulates the radiation environment of deep space. This aim has two purposes. If radiation leads to degradation of hematopoietic cell function it will clearly be of interest to look for the molecular lesions potentially responsible for such damage. Alternatively, more long term, but initially occult damage may also be induced. The consequences of such damage could lead either to a complete failure of hematopoiesis (aplastic anemia) or the development of hematologic malignancies. Identification of such damage is therefore important, and;
3. Design potential countermeasures to obviate or negate cellular and molecular damage discerned during the course of carrying out Aims 1 and 2.

We expect both simple and more complex solutions to problems that might be identified during the course of this study. We suggest that prophylactic (pre-flight) harvest and storage of astronaut stem cells might be a safe, effective and relatively inexpensive mechanism for countering long-term damage to cells of the hematopoietic systems. Countermeasures, which might prove effective in combating damage encountered during flight, will also be developed and explored for their utility.

Earth-Based Applications of Research Project

The development of effective radiation countermeasures could have significant impact on Earth for civilians and military personnel alike. With regard to the civilian population, it is quite conceivable that results we obtain will be relevant to patients undergoing cancer chemotherapy and radiation therapy. In this regard, it is possible that our studies will provide reagents and strategies for helping to protect normal tissues from the collateral damage of anticancer treatments. It is also possible that results we generate will be relevant to radiation workers and members of the armed forces who may be exposed to radiation during the course of carrying out their respective duties.

RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	Alan M. Gewirtz, M.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Effect of Deep-Space Radiation on Human Hematopoietic Stem and Progenitor Cell Function

Project Executive Summary

Astronauts on long-term missions in deep space will be placed at risk from a variety of hazards. Some of these are known while others may be anticipated. Damage to hematopoietic stem cells as a result of radiation exposure is as an example of the latter. Our long-term goal is to identify and quantitate the risks of deep-space radiation to the human hematopoietic system, with particular emphasis on the hematopoietic stem cell. Stem cells are the ultimate source of both the blood and immune systems, and damage to these cells could have grave immediate and long-term consequences. At the same time, because these cells can be readily removed from the body, manipulated and stored, they are also unique candidates for countermeasures that might obviate, or totally negate, damage incurred to them.

Accordingly, this project will have three specific aims which support our long-term goal and these are to: 1] Investigate the cellular consequences of exposing human hematopoietic stem and progenitor cells to an environment which simulates the radiation environment of deep space; 2] Examine the molecular consequences of exposing human hematopoietic stem cells to an environment which simulates the radiation environment of deep space. This aim has two purposes. If radiation leads to degradation of hematopoietic cell function, it will clearly be of interest to look for the molecular lesions potentially responsible for such damage. Alternatively, more long term, but initially occult damage may also be induced. The consequences of such damage could lead either to a complete failure of hematopoiesis (aplastic anemia) or the development of hematologic malignancies. Identification of such damage is therefore important; and 3] Design potential countermeasures to obviate or negate cellular and molecular damage discerned during the course of carrying out Aims one and two.

We propose both simple and more complex solutions to problems that might be identified during the course of this study. We suggest that prophylactic (preflight) harvest and storage of astronaut stem cells might be a safe, effective, and relatively inexpensive mechanism for countering long-term damage to cells of the hematopoietic systems. Countermeasures which might prove effective in combating damage encountered during flight will also be developed and explored for their utility.

RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	Ann R. Kennedy, D.Sc.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Countermeasures for Space Radiation-Induced Myeloid Leukemia

Project Executive Summary

This research investigates mechanisms to resist malignancy in space with an emphasis on evaluation of certain nutritional supplements as countermeasures to protect against space radiation-induced leukemogenesis; these nutritional supplements include L-selenomethionine (SeM), vitamin C, vitamin E succinate, alpha-lipoic acid, Co-enzyme Q10 and N-acetylcysteine. Data obtained from our previous *in vitro* and animal studies supported by the NSBRI have demonstrated that the nutritional supplements to be evaluated in this project are effective in preventing radiation-induced oxidative stress and protecting cultured human cells against adverse biological effects induced by the types of radiation of most concern during space travel, i.e., highly energetic heavy charged particles (known as HZE particles) and protons.

This investigation will determine the efficacy of a combination of these nutritional supplements as a countermeasure against space radiation-induced acute myeloid leukemia (AML) using the CBA mouse model system. Two, two-year animal experiments are planned to determine the effect of supplement combination on the development of AML in CBA mice exposed to radiation with HZE particles (1-GeV or 5-GeV/nucleon iron ions) or protons; blood cell profiles and gene expression (of myeloperoxidase in the spleen and CD33 in the bone marrow) patterns will be monitored throughout the studies, and gene expression patterns of the oxidation resistance gene (OXR1) will be determined at the end of the studies.

In addition, several short-term animal and cell culture experiments will be performed to determine the effects of radiation treatment and nutritional supplementation on three selected surrogate endpoint biomarkers, which are the host bio-reduction capacity measured as plasma total antioxidant status, plasma protein carbonyl content and OXR1 gene expression in selected populations of white blood cells. The results of this study are expected to provide critical information about the feasibility and mechanism(s) of nutritional supplements in increasing the resistance to space radiation-induced malignancy.

RESEARCH AREA:	Immunology, Infection, and Hematology
PRINCIPAL INVESTIGATOR:	William T. Shearer, M.D., Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Space Flight Immunodeficiency
END DATE:	06/30/2004

Project Executive Summary

On January 14, 2004, the President of the United States announced a new vision for the National Aeronautics and Space Administration (NASA). NASA included these directives to implement a human program to explore the solar system and beyond, and to extend human presence across the solar system, starting with human return to the Moon by the year 2020 in preparation for human exploration of Mars and other destinations.

However, long-term interplanetary space flight, such as the journey to the planet Mars envisioned within the next few decades, poses substantial health risks for humans. Many space flight conditions - as tested in human and animal space travelers and in earthbound human and animal models of space flight - give ample evidence of compromised immunity and the inability to resist microbial infections. Perhaps the most serious physical threat to health in space is radiation that is composed of photons (X-rays), electrons (g-rays), protons, neutrons, and heavy metal ions.

Investigations of mice given either 3 Gy of radiation (protons or g-rays) demonstrated that by days 4 to 10 after irradiation, there were statistically significant decreases in CD19+ B cells, CD3+ T cells, CD4+ T cells, CD8+ T cells IL-2 secretion by activated spleen cells. Irradiated animals showed a delayed and lowered anti-sRBC antibody response. Natural killer cells were relatively radioresistant. B cells recovered by day 15 and CD3+ and CD4+ T cells by day 29, but CD8+ T cells remained impaired.

As has been learned in transplant medicine, reactivation of latent viruses from within the host pose a formidable threat when host immunity is compromised. Latent viruses shedding has been shown to increase in humans in space shuttle flights (Epstein-Barr virus; cytomegalovirus) and in the Antarctic winter-over model of space flight (polyomavirus, herpesvirus). These viruses are known to be associated with human and animal cancers.

Original Aims of Project:

The hypothesis being addressed in our studies reported here is that space flight radiation will suppress the human immune system leading to reactivation of latent viruses, increased viral infections and disease, and the development of cancers. Since we can never purposefully test this hypothesis in humans, we use a murine animal model of g-ray and latent polyomavirus (PyV) infection to determine harmful effects upon the immune system with reactivation of latent virus infection.

Key Findings of Project:

Groups of BALB/c female mice were given whole body irradiation (3 Gy ¹³⁷Cs) or sham irradiation on day 0 and 49, and murine polyomavirus (PyV) or saline control on day 1: A, 3 Gy + PyV; B, no Gy + PyV; C, 3 Gy + no PyV; and D, no Gy + no PyV. Mice were tested for PyV replication by quantitative PCR, spleen weights and cell counts, and proliferation and gamma interferon (IFN- γ) production were measured at various intervals up to 69 days. Group A showed elevated PyV replication on days 10 and 20, as compared to Group B. Both Groups A

and B cleared PyV by day 49 in A and 20 in B. Only Group A again showed PyV replication when given a second dose of radiation on day 49. Spleen weights and cell counts of Group A were significantly lower than other groups. Irradiation suppressed T-cell proliferation in Groups A, B and C except in Group B when PyV was cleared. PyV infection enhanced IFN-g in all Groups: B > A > C.

Impact of Findings on Hypotheses:

This model of space flight suggests that the combined effects of radiation and latent virus infection will severely affect T-lymphocyte mediated immunity that may lead to chronic viral infection and malignancy. Thus, these findings partially validate the hypotheses, complete the objectives, and reply to the specific aims of the original project.

Proposed Research Plan for Coming Year

Our plan for the coming year is to publish at least one, possibly two, manuscripts of this research in a high-quality, peer-reviewed science journal. Also, because of the extraordinary expense of animal work, we plan to turn to an in-vitro B cell model to investigate the effects of radiation and countermeasures that could reverse immunosuppression. We will be able to perform many more experiments at a fraction of the cost of the animal experiments. When we have more information on mechanisms of apoptosis and its reversal, we plan to return to the animal model for selected experiments.

Earth-Based Applications of Research Project

Humans undergoing multi-modal immunosuppression for organ or bone marrow stem cell transplantation occasionally (i.e., 2% occurrence) develop polyclonal activation of B cells due to the latent Epstein-Barr virus (EBV). Repetitive activation of B cells by EBV may lead to the rapid growth of B cell clones that undergo malignant transformation. The B cell lymphomas are now treated with two forms of immunotherapy—EBV-specific autologous T cell clones, and anti-B cell monoclonal antibody (Rituximab). These are treatments that could be quantitatively explored in the murine immunosuppressive model of radiation and latent virus infection. Use of this model would permit the animal trials of additional modes of immunotherapy, such as the use of designer fusion proteins containing tumor-specific antibody and B-cell toxic reagents. Thus, this space research on irradiated and virus-infected mice may yield valuable information for the treatment of lymphomas in humans.

RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	Yufang Shi, D.V.M., Ph.D.
ORGANIZATION:	University of Medicine and Dentistry of New Jersey Robert Woods Johnson Medical School
PROJECT TITLE:	Effects of Antiorthostatic Suspension on the Immune System

Project Executive Summary

Hindlimb unloading (HU) is a well-accepted ground-based rodent model used to simulate some of the conditions of space flight and reproduce the deleterious effects on the musculo-skeletal, cardiovascular and immune systems. We are exploring the effects of HU on lymphocyte homeostasis and immune responses in the spleen and thymus of mice.

HU was found to drastically deplete various cell populations in the spleen and thymus. These changes are likely to be mediated by apoptosis, since DNA strand breaks indicative of apoptosis were detected by TUNEL staining in both splenocytes and thymocytes. Administration of opioid antagonists or interference with the Fas-FasL interaction blocked HU-induced reductions in splenocytes, but not thymocytes. On the other hand, a steroid receptor antagonist (RU486) blocked the reduction of lymphocyte numbers in both the spleen and thymus. Therefore, the effects of HU on the homeostasis of splenocytes and thymocytes must be exerted through distinct mechanisms. Stress by HU also severely curtailed antigen-specific splenocyte proliferation following immunization. The delayed-type hypersensitivity immune response measured by footpad swelling in response to secondary exposure to ovalbumin was decreased by HU. Similarly the expansion of ovalbumin-specific T cells following 2 doses of antigen was blocked by HU stress.

These findings demonstrate that lymphocytes are drastically affected by HU stress, and normal immune responses are severely blunted. These effects are mediated at least in part by apoptosis, and the Fas-FasL interaction is central in affecting lymphocytes in the spleen, but not the thymus. We performed a detailed analysis of splenocytes from mice subjected to 2 days of HU. Cell subpopulations were analyzed by staining with antibodies against specific lymphocyte markers: CD19, CD3 and CD56 to identify B cells, T cells and NK cells respectively, and CD4, CD8 and CD25 to distinguish T cell subpopulations. B cells were most significantly affected, although all major populations were reduced. Surprisingly, HU-treated mice showed significantly increased percentages of CD4+CD25+ cells, while their absolute numbers were unchanged. Therefore, these CD4+CD25+ are resistant to reduction by the stress of hindlimb unloading.

CD4+CD25+ T cells have been shown to play a central role in regulating immune responses and thus are termed regulatory T cells (Treg). Although much is known about the role of these cells in suppressing immune responses, little is understood about the function of CD4+CD25+ Treg cells in maintaining lymphocyte homeostasis. We found that depletion of CD4+CD25+ Treg cells in vivo by administration of anti-CD25 antibody dramatically prevented HU-induced lymphocyte reduction. This novel finding led us to conclude that CD4+CD25+ Treg cells play a critical role in lymphocyte homeostasis under situations of stress.

We also examined the effect of HU on T cell proliferation in vitro. Splenocytes isolated from control and stressed mice were activated with anti-CD3 and their proliferation assessed by ³H-thymidine incorporation. We found a significant reduction in the proliferation of T cells from mice subjected to 2-days of HU. When CD4+CD25+ Treg cells were selectively eliminated in vitro using anti-CD25 antibody, T cell proliferation recovered almost to the same level as controls. Thus CD4+CD25+ Treg cells are critical in down-regulating immune responses following hindlimb unloading.

It is difficult to quantify the degree of stress experienced by an individual since there is no specific stress marker. We recently employed proteomics technology and compared the serum protein composition of mice before and after HU treatment. We found that the serum level of transferrin was consistently increased many fold after HU. A few other HU-boosted proteins are currently being characterized. We believe that further study of these proteins may provide a marker to measure stress quantitatively. Further exploration of the mechanisms of stress-induced lymphocyte apoptosis and immunosuppression will allow for the development of more effective countermeasures for protection of astronauts and lead to medical applications here on Earth.

Earth-Based Applications of Research Project

Our findings have the following potential Earth-Based applications.

1. Manipulation of CD4+CD25+ cells should lead to a better understanding of the mechanisms by which stress affects the immune system and lead to potential interventions to prevent the deleterious health effects;
2. Identification of stress-regulated serum proteins could provide a marker to better measure stress levels in medical or occupational contexts, and;
3. The role of cellular apoptosis in stress-induced immune effects can be adapted for maintaining immune tolerance in medical practice.

RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	Gerald Sonnenfeld, Ph.D.
ORGANIZATION:	Morehouse School of Medicine
PROJECT TITLE:	Suspension, the HPA Axis and Resistance to Infection
END DATE:	05/31/2004

Project Executive Summary

This project was designed to determine the effects of space flight conditions expressed in the hindlimb unloading model for rodents on resistance to infection. Additionally, the role of the neuroendocrine system in regulating resistance to infection in this system was to be determined.

We have been able to show that hindlimb-unloaded mice had decreased resistance to infection with gram negative bacteria then did control mice. We were also able to show that pretreatment with Active Hexose Correlated Compound (AHCC), a nutritional supplement, protected mice from infection. The AHCC appeared to act by enhancing immune responses in the hindlimb-unloaded mice. The same bacteria were shown to have enhanced growth in the presence of catecholamines, which suggested that the stress response occurring in the hindlimb unloaded mice could contribute to the decreased resistance to infection observed.

The specific aims of the study were:

A) To expand the range of infections altered by AOH suspension. We had already shown that resistance to some infections that are not likely to be risks during space flight was altered by AOH suspension. We now determined that infections that could be a risk during space flight are affected by the suspension model.

B) To determine the mechanism of alteration of resistance to infection induced by AOH suspension. Although previous studies had shown that immune responses are altered by space flight, we now extended these studies to determine the role of the neuroendocrine system in regulating infections. This was carried out using two approaches. The data obtained from experiments using both approaches was integrated to allow for development of a model for the mechanism(s) of the effects of hindlimb suspension on resistance to infections.

Earth-Based Applications:

Our development of AHCC as a potential countermeasure for immune dysfunction induced by space flight has led to a new grant from the Amino Up Chemical Company to study the use of AHCC to prevent infection during severe trauma.

RESEARCH AREA:	Immunology, Infection and Hematology
PRINCIPAL INVESTIGATOR:	Gerald Sonnenfeld, Ph.D.
ORGANIZATION:	Binghamton University – State University of New York
PROJECT TITLE:	Bed Rest and Immunity

Project Executive Summary

Exposure of humans to space flight conditions results in changes in immune responses. The contribution of these changes in the immune system to alterations in resistance to infection and tumors remains to be fully established. Chronic bed rest with a head-down tilt has been a human model of choice for the effects of space flight on physiological systems. This model allows for no load-bearing on the legs of the subjects as well as a fluid shift to the head. Results utilizing the head-down tilt bed-rest model to study the effects of space flight conditions on the immune system are limited, but show a pattern similar to the results observed when humans are exposed to space. The head-down tilt bed-rest model has been established as valid for studying the effects of space flight conditions on functional dynamic immune responses such as cytokine production, but all of the studies to date have been carried out on men.

The study is designed to determine the effects of bed rest on immune responses of women. The overall hypothesis to be tested is that maintenance of female subjects in the head-down tilt bed-rest model will result in suppression of functional immune responses and enhance susceptibility to infection. The specific aims of the study are to determine effects of maintenance of female subjects in the head-down tilt bed-rest model on functional immune responses and on resistance to infection. By carrying out these studies, we will correlate the effects of bed rest on immune function and control of viral infections. The results of this study should aid in planning future space flight missions and development of countermeasures. This research is at countermeasure readiness level two.

**NSBRI RESEARCH PROGRAM
MUSCLE ALTERATIONS AND ATROPHY**

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Associate Team Leader:	Goldberg, A. L.	Harvard		
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RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Parker B. Antin, Ph.D.
ORGANIZATION:	University of Arizona
PROJECT TITLE:	Calpains in Simulated Microgravity-Induced Muscle Atrophy
END DATE:	06/30/2004

Project Executive Summary

The overall goal of this project is to test the hypothesis that inhibition of calpain activity in skeletal muscles can reduce myofibril degradation and muscle atrophy. Muscle wasting is an important impediment to extended space travel, and studies have shown that muscle size is regulated by the balance between myofibrillar protein synthesis and degradation.

Calpain is the major calcium activated protease in animal cells and plays a primary role in regulating the rate of muscle protein accumulation. Considerable evidence suggests that increasing the levels of calpastatin, a protein inhibitor of calpains, enhances muscle protein accumulation. Inhibition of calpain activity, either by increasing calpastatin levels or by expression of dominant negative forms of calpain, may therefore reduce or inhibit muscle atrophy.

Research in this project will explore these possibilities and has the following specific aims:

1. Investigate whether targeted over expression of calpastatin will reduce skeletal muscle atrophy in transgenic mice using the hindlimb unweighting model, and;
2. Investigate the use of dominant negative forms of calpains to inhibit calpain activity and reduce skeletal muscle protein degradation and atrophy.

Studies will use either the muscle creatine kinase promoter or a fully characterized tetracycline inducible system to express calpastatin or mutated calpains in muscles of transgenic mice or in cultured L8 muscle cells. Muscles will be analyzed for changes in overall size, nucleus/cytoplasm ratio, fiber type, total protein accumulation and degradation rates, and accumulation of individual myofibrillar proteins. Information gained is expected to broaden our understanding of muscle growth and may suggest approaches for alleviating muscle atrophy in space and on Earth.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Kenneth M. Baldwin, Ph.D.
ORGANIZATION:	University of California, Irvine
PROJECT TITLE:	Role of Muscle Loading on Mechanisms of Protein Translation and the Impact on Unloading-Induced Atrophy
END DATE:	02/29/2004

Project Executive Summary

In this project our goals were two-fold:

1. Ascertain a better understanding of the cellular factors that cause muscle atrophy;
2. Develop a resistance training protocol that can successfully create an anabolic state in the muscle that could serve to counteract the catabolic state that is associated with models of muscle unloading.

Our findings suggest that in rapid muscle atrophy models, there are fundamental reductions in the ability of key skeletal muscle genes to transcribe the message and the cell machinery to translate the message into protein. This deficit occurs in the face of elevations in the protein degradation systems that cause the normal breakdown in these proteins. Such an imbalance results in net protein loss in the muscle, thereby causing the muscle cell to become smaller. Resistance training improves the status of the muscle by slowing down the protein breakdown process while at the same time enhancing the muscle to synthesize new contractile protein. We are confident that by selecting the right prescription of resistance training, we will be successful in preventing the muscle wasting that occurs in space travelers.

We have identified useful and practical resistance training modes that improve the size of the muscle. Furthermore, we have identified likely processes impacting the imbalance in protein mass that occurs in response to skeletal muscle unloading. We have also identified the importance of growth factor expression that affects protein translational processes, which are important in maintaining positive protein balance in the face of unloading states. The goal is to use this information and design a resistance training prescription that can be effective in preventing muscle atrophy in the next funding period 2004-2008.

Specific Aims

The primary aims were to contrast the effectiveness of different contraction modes for inducing hypertrophy of rodent skeletal muscle using a computer driven training device. Muscles were activated with 40 contractions for each training session, with each contraction consisting of 2 seconds with appropriate rest intervals between each contraction and each set. The modes of contraction were of the isometric type, concentric type, e.g. shortening actions, and the eccentric type, e.g. lengthening actions of the same initial force generation. These experiments used muscle weight, total protein accumulation, insulin-like growth factor-1 and total RNA as key outcome variables.

Earth-Based Applications of Research Project

The problem of sarcopenia or muscle wasting associated with aging affects every human being over 40 years of age. Our research on the mechanisms of muscle atrophy and the use of resistance exercise, in the face of muscle unloading, will provide practical knowledge and useful strategies for ameliorating or slowing this critical health problem.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Kenneth M. Baldwin, Ph.D.
ORGANIZATION:	University of California, Irvine
PROJECT TITLE:	Force Regulation as a Countermeasure to Muscle Atrophy

Project Executive Summary

In recent years, considerable attention has focused on identifying exercise paradigms to ameliorate the deficits of atrophy and reduced strength in astronaut muscle properties, especially given that exposure to space flight is now minimally six months or longer on the International Space Station. While there is evidence that exercise of the resistance type is partially successful in reducing the muscle atrophy response, a definitive exercise prescription has not been identified that is suitable for conditioning skeletal muscle given the various constraints of microgravity that impact the homeostasis of astronauts.

The primary goals of the project are to utilize a rodent resistance training model in order to: 1) ascertain the efficacy of acute bouts of isometric, concentric, eccentric, and concentric-eccentric modes of contraction, as performed under conditions of muscle unloading, in their ability to activate molecular markers indicative of anabolic responses that have been shown to favor a positive protein balance profile in order to blunt the atrophy process; 2) test and optimize the most promising paradigm(s) for efficacy in ameliorating the atrophy of muscle associated with the model of hindlimb suspension; and 3) determine the efficacy of using resistance training, in combination with other pharmacological interventions (such as protease inhibitors), as to their ability to ameliorate muscle atrophy-induced processes as compared to resistance training alone.

Ultimately, the objective is to identify a resistance training prescription that can be efficiently performed (minimizing time constraints), requires simple, yet reliable equipment devices, and is attractive to the astronauts for compliance.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Vincent J. Caiozzo, Ph.D.
ORGANIZATION:	University of California, Irvine
PROJECT TITLE:	Hypergravity Resistance Training: Countermeasure to Microgravity

Project Executive Summary

A program priority of NASA's Biomedical Research and Countermeasures Program is to determine the potential usefulness of artificial gravity as a countermeasure, especially with respect to skeletal muscle atrophy and loss of muscle function. This project is a "proof-of-principle" of a unique countermeasure technology referred to as the "Space Cycle." The Space Cycle is a human-powered centrifuge that can be used to generate various levels of artificial/hypergravity. Artificial gravity/hypergravity in a microgravity environment could be used as a novel method of performing resistance training under high loading conditions. The novelty of artificial gravity/hypergravity resistance training is that each element of the body is loaded proportional to the local gravitational field, and under hypergravity conditions muscles like those of the leg can be made to work against very high loads (e.g., + 2 body weights) without the need for external weights.

The primary objective of this project is to use the Space Cycle to address the following general hypothesis: Artificial gravity can be used as a unique resistance training modality that acts as an effective countermeasure, preventing the loss of muscle mass and function that occurs due to microgravity. In addressing this issue, a logical sequence of experiments is proposed with the following objectives: 1) determine if squats performed under hypergravity conditions and without external weights can produce foot forces similar to those seen when performing squat resistance training under normal 1-G conditions; 2) determine if squats performed under hypergravity conditions produce muscle adaptations similar to those seen using a squat resistance training program under normal 1-G conditions; and 3) determine if a squat hypergravity resistance training program is an effective countermeasure to simulated microgravity. We are focusing on squat resistance training because squats recruit a broad spectrum of muscles in the leg and back, and are one of the classical exercises used by bodybuilders and athletes to hypertrophy muscles of the leg. Additionally, the so-called antigravity muscles of the leg are at the greatest risk for atrophy induced by microgravity. Furthermore, squats are a target exercise performed by astronauts on the International Space Station.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	P. Bryant Chase, Ph.D.
ORGANIZATION:	Florida State University
PROJECT TITLE:	Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle

Project Executive Summary

The overall goal of this project is to produce a muscle cell model (digital cell) that will: explain biomechanical adaptations that occur with alterations in muscle protein isoforms due to changes in activity level; predict bioenergetic changes associated with changes in activity level; and be integrated into computational models of human limb and heart. The essential molecular and subcellular components of the model will be identified and algorithms constructed based on experimental data obtained in a controlled environment. The cell model will be tested against published biomechanical and bioenergetic data obtained under a broad spectrum of environmental conditions. Our muscle cell model will be one of the main building blocks for constructing a model of integrated human function because the cell is the basic unit of physiological organization; the musculoskeletal system is ~80 percent of body mass and thus is a major determinant of energy consumption, as well as being responsible for movement and cardiovascular function. To accomplish our goal of constructing a digital muscle cell, we will: (1) identify contractile protein composition of skeletal and cardiac muscles from high- and low-activity rats; (2) characterize contractile properties (phenotype) of selected muscles containing unique mixtures of protein isoforms, as identified in Aim 1; and (3) develop the "digital" cell biomechanical model.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Alfred L. Goldberg, Ph.D.
ORGANIZATION:	Harvard Medical School
PROJECT TITLE:	The Activation of Protein Breakdown in Muscle Upon Unloading and Possible Countermeasures
END DATE:	02/29/2004

Project Executive Summary

Specific Aims

- 1) To clarify the mechanisms that activate the ubiquitin (Ub)-proteasome pathway during muscle atrophy induced by hind-limb suspension and by glucocorticoids which may also contribute to the muscle wasting in astronauts. We have made the surprising finding that one set of ubiquitination enzymes, E2_{14K} and E3 α , which comprise the "N-end rule" pathway, catalyze most of the ubiquitination in atrophying muscles. Therefore, we shall pursue studies to elucidate their special role and how this pathway is activated and contributes to muscle wasting.
- 2) To determine whether pharmacological inhibitors of the ubiquitin-proteasome pathway could be useful as countermeasures to reduce muscle proteolysis and atrophy and to synthesize novel types of inhibitors of this pathway.
 - a) We shall test whether inhibition of the "N-end rule" pathway might be an effective way to prevent atrophy through in vivo studies of transgenic or knockout mice with defects in E3 α or E2_{14K}. Related biochemical studies will attempt to identify more potent inhibitors of E3 α .
 - b) Our other approach toward countermeasure development will be to develop agents that partially inhibit proteasome function in muscle. Because available proteasome inhibitors can block its active sites completely, they are potentially dangerous and can only be used against life-threatening diseases. We recently discovered inhibitory sites in the proteasome by which certain peptides can feedback and retard (but not block) protein breakdown. We hope to synthesize safer types of inhibitors that function by this novel allosteric mechanism to reduce partially muscle protein degradation.
- 3) By using a gene microarray analysis, we hope to identify the spectrum of genes whose transcription rises or falls during muscle atrophy induced by hind-limb suspension or glucocorticoid treatments. Although several changes in transcription have been described in atrophying muscles, in order to fully understand the critical adaptations leading to the loss of mass and functional capacity, it is necessary to obtain a more complete picture of the changes occurring in muscle gene expression. Our initial experiments have already uncovered large increases in seven unidentified mRNAs. Such mRNAs could be useful markers to monitor muscle wasting and the efficacy of countermeasures. Also, identification of their functions could suggest new targets for pharmacological intervention. We shall also carry out a similar analysis of human muscle biopsies taken before and during prolonged bed rest (provided by W. Evans and coworkers) in order to test if insights gained from studies of the rodent models are applicable to atrophying human muscles.
- 4) To identify possible nonpharmacological approaches to reduce protein breakdown, we shall investigate the biochemical adaptations that occur in certain animals to suppress muscle

proteolysis and preserve muscle mass. We shall study muscles in two unusual physiological states: in black bears during winter (using biopsies provided by H. Harlow and coworkers) and in rats fed very low protein diets in which muscle protein is preserved despite disuse and decreased caloric intake. In addition, since muscle protein breakdown decreases in rats on protein-deficient diets, we shall test whether in such animals there is less atrophy upon hind-limb suspension.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Alfred L. Goldberg, Ph.D.
ORGANIZATION:	Harvard Medical School
PROJECT TITLE:	The Activation of Protein Breakdown in Muscle Upon Unloading and Possible Countermeasures

Project Executive Summary

The rapid loss of muscle mass that occurs in astronauts in space due to muscle unloading and in patients with many systemic diseases results primarily from accelerated degradation of muscle proteins. This enhancement of protein breakdown is mainly due to activation of the Ub-proteasome pathway. Our major goal is to clarify the biochemical basis for the activation of this proteolytic pathway and thus to develop pharmacological agents that reduce this excessive degradation and retard muscle wasting. Recently, we identified a set of genes ("atrogin") whose expression increases or decreases coordinately when muscles atrophy. In order to achieve a fuller understanding of the atrophy process, we plan to further study this transcriptional program and its regulation, especially after disuse.

Of particular importance was the recent finding that the two genes induced most dramatically in atrophying muscles are the muscle-specific ubiquitin ligases (E3s), atrogin-1 (MAFbx) and MuRF1. If either of these enzymes is knocked out, the extent of muscle wasting is reduced. These Ub-ligases thus are very attractive therapeutic targets. To fully understand the initiation of the atrophy process and to develop rational countermeasures, we are studying the signal transduction systems that activate transcription of these genes in simple models of muscle atrophy in cultured myotubes. We recently found that the key factor in muscle hypertrophy, IGF-1, rapidly suppresses the expression of atrogin-1 and MuRF1 and prevents their induction by glucocorticoids. These effects of IGF-1 appear to be mediated by the PI3-kinase-AKT pathway, which probably inactivates one of the Forkhead transcription factors. Our primary goals will be to identify the precise steps in this kinase cascade and the key transcription through which disuse and glucocorticoids activate and IGF-1 inhibits expression of atrogin-1 and MuRF1. We shall also examine whether other atrogins are regulated by the same signaling systems and transcription factor.

These studies should identify novel therapeutic targets (e.g. key kinases) whose inhibition blocks atrogin-1 and MuRF1 induction. Using these enzymes and "reporter gene" constructs, we shall screen libraries of small molecules for agents that prevent induction of atrogin-1 and MuRF1. Inhibiting the expression of these key ligases represents an exciting new therapeutic approach to prevent muscle wasting in space personnel and in diverse disease states.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Marc T. Hamilton, Ph.D.
ORGANIZATION:	University of Missouri
PROJECT TITLE:	Genomics of Human Skeletal Muscle During Bed Rest and Exercise

Project Executive Summary

Reduced use of weight-bearing skeletal muscles during microgravity and sedentary life on Earth causes unhealthy and potentially dangerous consequences. For example, leg muscles atrophy, and also have a profound reduction of lipoprotein lipase activity (an enzyme in the blood vessels of muscles with a protective effect against lipoprotein risk factors for coronary heart disease). It is likely that an unbiased determination of the global expression pattern of the human genome with microarrays will reveal many muscle mRNAs increasing and decreasing, including mRNAs that heretofore have never even been hypothesized to contribute to the "microgravity or sedentary phenotype." Additionally, large scale genomic studies are likely to begin to reveal clusters of related mRNAs that provide clues as to the sets of genes orchestrating some of the cellular signaling, transcriptional changes, cellular growth, and metabolism. This project will build upon recent experience established from microarray studies of hindlimb suspension, endurance exercise, and muscle fiber type that support the statements described above. The effects of bed rest and one-leg exercise (as a countermeasure to attenuate the effects of inactivity) on the soleus muscle of 6 men and 6 women will be studied. Using state-of-the-art microarray methodologies, this project will measure the expression of ~12,000 full-length sequence verified mRNAs and ~3,000 of the most abundant muscle ESTs. This project is being proposed by a laboratory already using microarrays in the study of muscle physiology, in collaboration with a bioinformatics laboratory, a physical therapy laboratory focused on muscle function, a physician-scientist studying muscle diseases, and a core laboratory for microarray development. This study is likely to discover novel candidate genes and clusters of related genes potentially responsible for the unhealthy responses to reduced muscle use during physical inactivity.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Susan C. Kandarian, Ph.D.
ORGANIZATION:	Boston University
PROJECT TITLE:	Gene Expression Profiling of Unloaded Skeletal Muscle
END DATE:	05/31/2004

Project Executive Summary

Original aims:

The overall goal of this project is to elucidate signaling mechanisms that mediate the adaptation of mammalian skeletal muscle to mechanical unloading. In identifying cellular mechanisms we will be in a better position to develop effective countermeasures. Global gene expression profiling was a major tool used to address this goal. The specific aims were:

- To conduct global gene expression analysis in mechanically unloaded mammalian skeletal muscle. Affymetrix RatU34A GeneChips were used to probe mRNA expression in rat soleus muscle after 1, 4, 7 and 14 days of hindlimb unloading.
- To identify candidate factors and pathways involved in the regulation of unloading induced muscle atrophy. Clustering algorithms were then used to elucidate sets of genes, with known or unknown functions, that are co-regulated based on temporal expression patterns. These approaches provided insight into possible gene associations and candidate players in the pathways that regulate the atrophy process, and thereby proposing them for further study. Quantitative analysis of candidate factors and pathways involved in regulating unloading induced atrophy.

Key findings of the project

The results from the initial phases of the project have recently been published in their entirety. In brief, expression of 309 known genes was significantly changed by at least 2-fold. K-means clustering was used to divide these genes into co-regulated clusters based on the similarity of temporal expression patterns. This allowed the development of a timeline of the atrophy process with respect to the behavior of genes in a broad array of functional categories. Regulatory genes were often upregulated early, in either a transient or sustained manner, but they also populated clusters with later patterns of activation, suggesting different phases of molecular adaptations. Other early events were the activation of ubiquitination genes and downregulation of protein chaperones. In comparison, clusters representing slightly delayed activation patterns included genes involved in fast contraction, glycolysis, translational inhibition, oxidative stress, protein degradation, and amino acid catabolism. Downregulated genes exhibited fewer unique expression patterns and included structural and regulatory genes of the extracellular matrix and cytoskeleton, and genes that define a slow phenotype. Other novel findings include the tight co-activation of proteasome subunit and ubiquitination genes, differential regulation of serine proteases and serine protease inhibitors, and the identification of transcriptional, signaling, growth and cell cycle genes that likely play a role in atrophy. The present work has uncovered temporal patterns of gene expression that highlight the molecular processes that underlie muscle atrophy and provide new avenues for study. Nedd4 project: Results from several laboratories have shown that the ubiquitin-proteasome system is responsible for the majority of muscle protein loss that occurs with disuse. Ubiquitin-protein ligases (E3s) are responsible for the targeting of specific proteins for degradation by the proteasome. Our microarray data reconfirm

that Atrogin1 and MuRF1 are upregulated during unloading, but we have also identified another upregulated ubiquitin-protein ligase not previously characterized with respect to muscle atrophy called Nedd4. Nedd4 has a pattern of activation very similar to that of Atrogin1/MAFbx. We have confirmed this pattern of activation at the mRNA and protein level. Nedd4 is known to ubiquitinate membrane proteins but its role in muscle protein turnover has not yet been defined. We are in the process investigating the role of Nedd4 in muscle using the tools described.

Impact of findings on objectives

The data suggests several pathways that are at work during muscle atrophy. The results also indicate that there are several different temporal switches of regulatory genes that are activated during atrophy. The next step towards studying the function of these putative regulatory genes is to overexpress or inhibit their actions in muscle cell culture or in vivo. In order to study the role of the candidate genes we have identified from our microarray analysis of atrophying skeletal muscle, we are using conditional expression systems or transduction of candidate genes post-differentiation in cell culture and in vivo. The approaches we are using for the overexpression or inhibition of candidate genes are: a doxycycline conditional expression system and an adenoviral expression system. For in vivo experiments we are using direct plasmid injection with electroporation. The vectors encode one of the several genes we will study in either wild-type, constitutively active, or dominant-negative form. We will then test the effects of the overexpression of these genes in whole muscle or in muscle cell culture using standard biochemical and morphological assays.

Earth-Based Applications of Research Project

It is well known that exposure to microgravity, as well as muscular unloading on earth, leads to marked decreases in muscle size, functional capacity, and fatigue resistance. What is poorly understood about atrophy is how the protein loss is controlled, including the triggers and signaling mechanisms. Results from the microarray analysis of unloaded rat soleus muscles have not only given us insight into the processes and pathways involved in muscle atrophy but they have provided an excellent source of candidate genes to test for regulatory roles in the atrophy process. In identifying these genes we will be in a better position to develop more effective countermeasures to combat the deleterious changes in muscle function due to exposure to spaceflight.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Martin J. Kushmerick, M.D., Ph.D.
ORGANIZATION:	University of Washington
PROJECT TITLE:	Integrating Human Muscle Energetics and Mechanics
END DATE:	05/31/2004

Project Executive Summary

Background

Human muscle performance depends on a number of factors beyond the mechanisms in neural activation and control. In fact, the experienced and trained nervous system depends on stable biomechanical properties of the muscle for skilled and reliable limb performance. The biomechanical properties depend on the size of the muscle (muscle quantity) and the phenotype of the muscle cells (muscle quality). Most of the other projects in the muscle team are concerned with the regulation of muscle mass (risk of wasting and atrophy).

This project uniquely analyzes by experiment and modeling the interplay between biomechanical properties and energy metabolism. That this is important is obvious from the facts and extensive literature on the sustainability of muscle performance and ease of fatigue. Decreased performance and fatigue is determined by an integration of muscle properties, mass of muscle and biomechanical demand. The first part of this project analyzes and quantifies the internal structures of human limb muscle, with the goal of translating external torque and length changes produced across a limb by a given muscle into the actual forces and lengths of the muscle cells experience when organized as fascicles. This work enables a cellular and mechanistic analysis well beyond what is currently done and described in the literature for human muscle.

Biomechanical power output must be matched quantitatively by biomechanical power input within the cells for sustained activity beyond a few tens of seconds. The second arm of this project applies quantitative measures of the components of energy balance (ATPases, glycolysis and oxidative phosphorylation) to measure the energetic properties of selected muscles and how these vary among individuals. These measurements are then analyzed in the third arm of this project by a quantitative model to obtain metabolic fluxes and in combination with mechanical data to obtain quantitative analyses of economy, efficiency or doing work and related parameters.

Goals

The overall goal of this project is to build up a series of measurements and models of:

- Intracellular energy and metabolic fluxes (ATP supply and demand);
- Mechanics during exercise (ATP demand); and
- Blood flow (ATP supply).

The goal will make possible an integration of the mechanisms involved and a validation of models describing these processes.

Aim One: Measure the economy and efficiency of human muscle contraction and sustainable power output.

Hypotheses tested:

1. The balance between ATP supply and ATP demands account quantitatively for the difference in sustained performance (duty cycle) in various muscles and individuals.

2. Working contractions add a substantially larger, myofibrillar cost above isometric twitches in which only ion transport activation costs dominate.
3. Muscles differ in economy and efficiency: mechanically slower muscles have higher economy and thermodynamic efficiency for converting ATP to external work than fast muscles, but lower power.

Aim Two: Integrate the component mechanisms of energy supply and demand into models to make predictions of energy costs and balance in new experimental conditions.

Hypotheses tested:

1. Simple models of the components of energy balance as developed in aim one are necessary and sufficient to account for the major energetic properties of human muscle.
2. The models establish a cellular basis for defining isometric economy and working efficiency.
3. Variations in properties among muscles and individuals define the normal distribution of properties so this distribution can be used to define probabilistic responses of the system.
4. When new mechanistic components at the molecular and cellular level are added, they can be tested for their effects on the system operation.

Relevance to Risk Reduction and Countermeasure Development

Even in the presence of significant atrophy muscle may be capable of sufficient and sustainable power output provided the muscle is operating over an appropriate portion of the force-velocity and power-velocity curve and provided that there is sufficient steady state and dynamic metabolic power. While not minimizing the importance of adequate muscle mass and the deleterious effects of muscle atrophy, we focus on the equal or more important aspect of the muscle functional properties in intact humans by entirely non-invasive methods, some of which can be conducted in the International Space Station and on long-duration expeditions.

Current risk assessment is based entirely on evaluating the consequences of decreased muscle mass. The information provided by this project enables an evaluation of the consequences (both positive and negative) of altered muscle performance. The modeling enables a forward analysis of altered exercise strategies to accommodate possibly the same motor tasks. It also enables a prediction of the biomechanical responses to the muscle phenotype that would be produced by countermeasures developed by molecular and pharmaceutical procedures being investigated in other projects of the team and by exercise strategies being developed by other teams and by JSC and other NASA professionals. It is likely that the bioenergetic/biomechanical analyses would be useful in design of space suits because of the inevitable addition of mass, friction and viscosity of the suit to the overall limb function.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Michael B. Reid, Ph.D.
ORGANIZATION:	University of Kentucky Medical Center
PROJECT TITLE:	Redox Modulation of Muscle Function in Microgravity

Project Executive Summary

Exercise-induced fatigue and muscle atrophy are mediated in part by reactive oxygen species (ROS), a stimulus that may be exaggerated by radiation during spaceflight. We are assessing the roles of ROS signaling and radiation on muscle fatigue and atrophy, and testing antioxidants as possible countermeasures.

Specific Aims:

Aim 1: To determine if oxidative stress contributes to muscle fatigue during handgrip exercise.

- Fatigue of hand and forearm muscles may limit crew performance during extravehicular activity (EVA). N-acetylcysteine (NAC) is an antioxidant that inhibits muscle fatigue in humans. We have recently completed experiments testing the capacity of NAC to inhibit muscle fatigue and oxidative stress in humans during handgrip exercise. Working with Dr. Jeff Jones, Flight Surgeon at NASA Johnson Space Center, we used equipment and test procedures designed for use on the International Space Station. Results of the study show the feasibility of this approach. NAC abolished glutathione oxidation blood draining the affected muscle groups and increased handgrip endurance by 30% relative to untreated trials. Follow-up studies are being planned that will test the importance of these findings in a more operationally-relevant setting.

Aim 2: To determine whether ionizing radiation accelerates ROS production and fatigue in skeletal muscle.

- We postulated that proton radiation absorbed during EVA would increase tissue ROS levels and accelerate muscle fatigue. We were testing this postulate in collaboration with Dr. Carlos Gonzalez, Director of the cyclotron at the University of Texas Medical School, when Tropical Storm Allison destroyed the cyclotron facility in 2001. The facility has not been rebuilt. Resources intended for these experiments have been redirected to studies of mechanisms regulating muscle atrophy and to tests of potential countermeasures (Aim 3).

Aim 3: To evaluate oxidative stress as a mediator of muscle weakness caused by gravitational unloading.

- Muscle atrophy and contractile dysfunction cause weakness after prolonged spaceflight. We are evaluating oxidative stress as a cause of these changes in mouse soleus during 12-days of hindlimb unloading and are using cell culture techniques to evaluate mechanism. Our results show that:
 1. Unloading increases oxidant activity within soleus muscle fibers;
 2. Contractile dysfunction is blunted by administration of some antioxidants (NAC, allopurinol) but not others (curcumin, vitamin E);
 3. A novel ubiquitin conjugating enzyme, UbcH2/E220k, is highly expressed in skeletal muscle, is upregulated by ROS exposure, and mediates ubiquitin conjugation to muscle proteins;

4. Hydrogen peroxide upregulates expression of atrogin1/MAFbx, a key ubiquitin ligase that regulates muscle atrophy;
5. This signal is transduced by p38 MAP kinase; and
6. p38 inhibition blocks atrogin1/MAFbx upregulation and the associated rise in ubiquitin conjugating activity. We are testing the roles of these transcriptional mechanisms in atrophy of unloaded muscle and will continue to evaluate potential countermeasures.

Aim 4: To determine if radiation stimulates atrophic signaling in muscle.

- We postulated that radiation-derived ROS might stimulate catabolic signaling and planned to measure activity of redox-sensitive pathways in muscle after proton irradiation. Destruction of the Medical Center cyclotron has prevented these experiments. Project resources have been redirected to studies of cellular mechanism and putative countermeasures (Aim 3).

Earth-Based Applications of Research Project

This research directly addresses two Earth-based problems, loss of function in unloaded muscle and muscle fatigue. The first problem occurs in individuals who are immobilized by injury or surgery. Muscles of the affected limbs atrophy and weaken, making it difficult for the individual to return to normal daily activity. The resulting inactivity lessens the quality of life, increases hospitalization and therapeutic costs and increases the likelihood of pneumonia, venous thromboses and other serious medical complications. A practical countermeasure to lessen atrophy and weakness would directly benefit these individuals, lessening the problems caused by transient immobilization.

The second problem is familiar to us all. Acute muscle fatigue is a common feature of strenuous exercise. A countermeasure to inhibit fatigue would benefit a broad range of the US populace whose work requires physical exertion ranging from military professionals to firefighters, from police officers to construction workers. [The implications for professional athletes are all too obvious.]

RESEARCH AREA:	Muscle Alterations & Atrophy
PRINCIPAL INVESTIGATOR:	Shantanu Sinha, Ph.D.
ORGANIZATION:	University of California, Los Angeles
PROJECT TITLE:	InVivo Stress-Strain Dynamics in Human Muscle

Project Executive Summary

Unique Claims of Study:

One of the most significant problems facing astronauts is that of muscle atrophy resulting from microgravity or immobilization during space flights. Methods that can reveal the functional status of different muscle groups, in a robust, clinical and preferably non-invasive and safe manner, could have a significant clinical impact in the physical well being of the astronaut. Knowledge about how the strain distribution, muscle volume and the maximum force the subject can exert, change as atrophied muscle progresses towards normalcy can be a valuable clinical parameter to quantify the relative efficacy of different therapeutic strategies for rehabilitation used for astronauts, and can help one understand the complex interactions between active contractile tissues and passive connective ones. The method we have developed, using phase contrast, velocity encoded MR imaging technique is such a non-invasive, non-ionizing and safe method that can be used routinely in the clinic. We have been able to prove its efficacy in monitoring the changes after onset of atrophy and the method is sensitive enough to follow the changes as the muscles return to normalcy as the patient is given rehabilitative physiotherapy.

Specific Aims:

- Develop a non-invasive method to estimate quantitatively the extent of atrophy in human lower leg muscle complex, the reduction of maximal force the subject can exert after atrophy sets in, and the extent of degradation of functionality of different muscle groups.
- To induce atrophy in the leg of human subjects by Unilateral Limb Suspension and use the above method to estimate how those parameters are affected by this controlled atrophy.
- Similarly to measure those parameters when atrophy results from immobilization from casting following surgery in patients with Achilles Tendon Rupture.
- To use this method to acquire data with an accuracy hitherto not available, in order to better understand the correlation between structure and function of the different muscle groups within the human triceps surae complex.

Main Findings in Reporting Year:

- A completely non-invasive method was developed and perfected using safe and painless, velocity encoded, phase contrast MR imaging techniques, to measure the effect of muscle atrophy in the human lower leg. The parameters that could be measured and quantified included total volume of different muscle compartments, the maximum torque that the subject could exert and finally, and most importantly, the contraction velocity at any arbitrary point in leg, within any muscle group during an isometric contraction cycle. All three parameters are affected by muscle atrophy.
- The above methodology was first applied to 10 normal subjects, in whom atrophy was induced by suspending one leg for a period of four weeks. The above method was very

effective in showing consistent patterns of changes in all three parameters, muscle volume, force of MVC and the contraction velocity. The method could detect differential changes in different muscles groups, as well as track the return to normalcy as the subjects were given physical rehabilitation/therapy.

- Similar changes in muscle function (contraction velocity), muscle volume and strength could be quantified and tracked in 3 patients who had undergone surgery to repair Achilles tendon rupture, with consequential muscle atrophy. These changes were further tracked during six weeks of re-ambulation and physical therapy. Once again, consistent patterns of changes could be detected in all three parameters.
- The structure of human multi-pennate soleus muscle in vivo as elucidated by high resolution MRI and 3-D volume rendering was correlated with the heterogeneous spatial distribution of functionality, determined in terms of peak shortening velocity during isometric contraction using the above method.

Earth-Based Applications of Research Project:

The method developed herein, namely, using a non-invasive method of monitoring structural and functional differences between normal and atrophied muscles has a tremendous potential for Earth-based clinical use. Atrophy can result not only from micro-gravity as in the case of astronauts in space flights, but also from prolonged bed-rest or immobilization from casting. In these cases, the extent to which the muscle normalizes subsequent to rehabilitation is a very important clinical question, and can be addressed quantitatively by the method developed herein.

RESEARCH AREA:	Muscle Alterations and Atrophy
PRINCIPAL INVESTIGATOR:	Robert W. Wiseman, Ph.D.
ORGANIZATION:	Michigan State University
PROJECT TITLE:	Ca⁺² Homeostasis and Muscle Phenotype: Role of Cellular Energetics

Project Executive Summary

Exposure of skeletal muscle to space flight results in a significant loss of mass and a shift in the phenotype from slow to fast muscle isoforms. To a limited extent, astronauts are able to ameliorate this remodeling of muscle tissue through exercise. If the mechanistic link between physiologic function and phenotype were better understood, design of countermeasures using combinations of exercise protocols and pharmaceuticals could be employed to increase the efficacy of training while on space missions. We propose that altered physiologic function signals the initiation of the remodeling process through Ca⁺² sensitive transcription factors (CSTFs) which are activated through changes in two homeostatic processes; mitochondrial ATP synthesis and sarcoplasmic reticulum (SR) ATPase Ca⁺² handling. It is our assertion that alterations in phenotype in response to changes in load bearing or any other metabolic stress involves processing information from the physiology in the form of feedback from these two homeostatic processes. We use an integrative approach to study this problem in isolated superfused skeletal muscles using a combination of non-invasive techniques (³¹P-NMR and fluorescence spectroscopy and mechanics) and molecular techniques. In the first Aim we determine the sensitivity of cytosolic Ca⁺² handling to metabolic loads induced by electrical pacing and metabolic inhibitors. In the second aim we test the response of CSTFs to alterations in Ca⁺² homeostasis using ionophores, SR ATPase inhibitors as well as the metabolic stresses we develop in Aim 1. We believe once the mechanistic link is established that we may be able to design countermeasures to mask the loss of mechanical loading by direct manipulation of cytosolic Ca⁺² and more effectively stave off the changes occurring in limb musculature.

**NSBRI RESEARCH PROGRAM
NEUROBEHAVIORAL AND PSYCHOSOCIAL FACTORS**

Team Leader:	Dinges, D. F.	Penn		
Associate Team Leader:	Brady, J. V.	Johns Hopkins		
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RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Gary Aston-Jones, Ph.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Stress, Performance and Locus Coeruleus
END DATE:	08/31/2004

Project Executive Summary

Original Aims

The original aims of this project were: 1. Analyze the activity of brain stem noradrenergic locus coeruleus (LC) neurons during a continuous performance task, 2. Determine the effects of acute and repeated stress on changes in LC function and performance, and 3. Identify pharmacological countermeasures to mitigate stress effects on LC activity and attentional function.

Key Findings

1. *Development of a continuous performance task for the rat.* We developed a target detection continuous performance task that rats can learn rapidly. This task mimics many of the attributes of the target detection task in our previous studies in monkeys in which LC activity appears to play a major role. Rats initiate each trial by pressing one lever, and then must discriminate between two signal lights to determine if the one illuminated is a target or non-target. If the target signal light is illuminated the rat must press a second lever to obtain food reward. If the non-target is illuminated he must withhold responding with no reward and await the next trial. Targets occur randomly on 20 percent of the trials. This task will be the means by which we measure performance abilities and changes therein induced by stress and pharmacologic treatments.

2. *Effects of stress on performance in the target detection task.* To date we have tested only acute noise stress on performance of this task. Results indicate that white noise during task performance at 90 db significantly increased responding to the non-target stimulus (false alarm (FA) errors) in this task. Interestingly, this effect habituated rapidly, so that subsequent administration of 105 db did not influence performance. Chronic stress (planned for this year) may be needed to see continued performance deficits. The alpha2 adrenoceptor agonist clonidine (which decreases LC-NE neurotransmission) at 8 mg/kg reduced the FA error rate seen with 90 db noise stress. Higher doses of clonidine (25 mg/kg) produced sedation. These preliminary experiments require confirmation with additional studies, but they suggest that the NE system may be involved in stress effects on performance in this task.

3. *Effects of idazoxan on performance in the target detection task.* The alpha2 adrenoceptor antagonist idazoxan increases firing of LC neurons and release of NE from LC terminals. Our view of LC's role in performance predicts that this agent should worsen performance on this task, with increased FA errors (as observed in monkey LC neurons during periods of high tonic LC activity). Systemic idazoxan had no effect on two rats that were performing marginally in the task (i.e. a 30 percent false alarm rate). However, this compound markedly increased false alarms in both of the rats that were performing exceptionally well and had low baseline false alarm rates in the absence of the drug. Although preliminary, these results are consistent with the view that moderate levels of tonic LC activity are critical for maintaining focused attentiveness to task stimuli and performing optimally, and that behavioral performance declines when tonic LC firing rates are increased. We speculate that the lack of an effect of idazoxan in rats with marginal baseline performance reflects the inverted U relationship described by the PI for the relationship between LC activity and performance on such a task. Thus, in these rats the poor

performance pre-drug may have been due to a high level of baseline tonic LC activity, placing them at the right of the inverted U relationship. This pre-existing heightened LC activity could have created a ceiling effect that prevented idazoxan from further increasing LC firing rates and disrupting responses.

4. **Role of the LC in circadian regulation of sleep and waking.** We expanded our program to include analysis of the role of LC in effects of sleep deprivation on performance. We took this step because sleep deprivation is one of the largest stresses affecting the astronaut, and there are well-established effects of sleep deprivation on performance. For this, considerable technological development has occurred. We implemented a telemetry system for recording EEG, EMG, body temperature and locomotor activity in freely moving, untethered rats. This system produces robust sleep measures over long periods of time. We have also developed a mechanism for producing sleep deprivation, consisting of a slowly rotating wheel that the rat is within. This device allows access to food and water and also contains levers and stimuli to allow task performance during the sleep deprivation period. We will use this system to deprive rats of sleep at different times of their circadian rhythm and examine effects on performance. We will then analyze effects of manipulating the LC system on the performance deficits produced by sleep deprivation.

Impact of findings

The development of a target detection task for the rat now allows us to test the effects of stressors on a type of performance important in space missions. This model will also allow analysis of the effects of manipulations of the brain NE system of the LC in these stress effects to facilitate development of countermeasures that should facilitate performance in the face of stress. We found that acute stress increases FA errors in this task, and that decreasing neurotransmission in the LC system with clonidine may offset this effect. Accordingly, we also found that increased NE neurotransmission (with idazoxan) in non-stressed animals worsens performance on this task by producing the same type of errors (FAs). These results indicate that the LC-NE system may be a valid target for development of countermeasures to the effects of stress on performance. Finally, we have developed a device to sleep-deprive rats and measure effects on performance in this task. This will allow analysis of this important stressor on performance, and the ability of manipulations of the LC system to offset such stress effects on performance.

Proposed research plan for next year

Studies will continue in the areas described above to confirm findings to date. In addition, new studies will be undertaken to examine the effects of chronic noise stress, as well as sleep deprivation stress, on performance. The ability of manipulations of the LC-NE system to offset these stress effects on performance will be determined.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Joseph V. Brady, Ph.D.
ORGANIZATION:	Johns Hopkins University School of Medicine
PROJECT TITLE:	Psychosocial Performance Factors in Space Dwelling Groups
END DATE:	02/29/2004

Project Executive Summary

Despite uncertainties regarding the performance requirements of projected space laboratories, work stations, interplanetary vehicles and settlements beyond the Earth's atmosphere, a common feature of spaceflight endeavors over the next half century will be extended stays by human groups in extraterrestrial habitats. The imperatives and opportunities associated with configuring effective psychosocial performance models in support of such space dwelling groups are best served by research approaches that are both heuristic and innovative. The development of functional human and ecological models for such space dwelling groups must, in the first instance, be based upon sound scientific principles with research objectives focused upon the management of semi-permanent as well as permanent groups involved in both operational and space science missions. The research methodology involves development of a distributed interactive multi-person simulation in computer generated environments as an experimental test bed for modeling psychosocial performances within and between space-dwelling and Earth-based groups. The simulation approach provides an automated means of setting the context for the analysis of performance in space-dwelling groups and monitoring electronically the effects of varying experimental conditions that alter psychosocial interactions.

Distributed interactive simulation experiments characterize the effects of variations in the structure and function of communication channels within and between space-dwelling and Earth-based groups as well as the effects of stressful environmental and behavioral interactions upon psychosocial performance effectiveness. Simulation experiments also determine the effects of variations in the appetitive/aversive characteristics of incentive control systems as well as the effects of selection, training, and experience within and between space-dwelling and Earth-based groups. Communication modes, frequencies, durations, and content are recorded and analyzed with performance effectiveness evaluations based upon assigned group task outcome measures. Conceptual and methodological advances that effectively promote psychosocial and ecological stability will ultimately benefit larger societal units, including those that remain Earth-bound, by enhancing an educational and training technology that assures communication of an expanded generalizeable knowledge base. The results of these studies with scenario tasks requiring identification of geologic samples designated by five different rules in each region, showed clearly that cooperative and productive psychosocial interactions could be maintained between individually isolated and dispersed crewmembers in the simulated task environment.

All experimental flight crews actively engaged in communicating and effective problem-solving over extended time intervals without benefit of one another's physical presence. In addition, the investigations of communication modality constraints indicated that, with the scenarios tested, there was a high degree of interchangeability between the available communication modes. For example, the effect of selectively removing text or audio communication was to increase the number of audio messages somewhat when text was removed, and produce a clear increase in

text messaging when audio was removed. The overall performance evaluation however, showed no consistent effect upon crew total grade values of eliminating either text or audio. This suggests a high degree of functional interchangeability between these two communication modalities. Removal of both audio and text however, produced a marked decrease in overall performance effectiveness. Although there was a commensurate increase in the use of whiteboard scribbles, total crew grade values declined to less than half the baseline in the combined absence of audio and text communication modalities.

Earth-Based Applications of Research Project

Research conducted within the context of distributed interactive simulation models will provide the basis for developing effective patterns of communication and problem-solving strategies as well as a range training procedures to enhance problem solving effectiveness. The Earth-based applications of this research will extend to the small operational group selection and training process as well as the management of stressful interactions and the maintenance of group cohesion and productivity. Not only can the outcome of these studies be expected to have an important impact on safety and the quality of life in many Earth-based applied settings, but larger societal units will ultimately benefit from the resulting conceptual and methodological advances that effectively promote social and ecological stability while concurrently enhancing an education and training technology that assures effective communication of an expanded, generalizeable knowledge base.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Joseph V. Brady, Ph.D.
ORGANIZATION:	The Johns Hopkins University School of Medicine
PROJECT TITLE:	Psychosocial Performance Factors in Space-Dwelling Groups

Project Executive Summary

Despite uncertainties regarding the performance requirements of projected long-term space laboratories, work stations, interplanetary vehicles and settlements beyond the Earth's atmosphere, a common feature of space flight endeavors over the next half century will be extended stays by human groups in extraterrestrial habitats. The imperatives and opportunities associated with configuring effective psychosocial performance models in support of such space-dwelling groups are best served by research approaches that are both heuristic and innovative. The development of functional human and ecological models for such space-dwelling groups must, in the first instance, be based upon sound scientific principles with research objectives focused upon the management of semi-permanent as well as permanent groups involved in both operational and space science missions.

The research methodology for this continuation research project will involve the application of a distributed interactive multi-person simulation in computer-generated environments developed during the initial years of project support as an experimental test bed for modeling psychosocial performances within and between space-dwelling and Earth-based groups. The simulation approach provides an automated means of setting the context for the analysis of performance in space-dwelling groups and monitoring electronically the effects of varying experimental conditions that affect psychosocial interactions.

Distributed interactive simulation experiments characterizing the effects of variations in the structure and function of communication channels within and between space-dwelling and Earth-based groups as well as the effects of stressful environmental and behavioral interactions upon psychosocial performance effectiveness have been undertaken and partially completed during the initial years of project support. Simulation experiments will continue to determine the effects of variations in the appetitive/aversive characteristics of incentive control systems as well as the effects of personality variables, selection, training, and experience on performance interactions within and between space-dwelling and Earth-based groups. Communication modes, frequencies, durations and content will continue to be recorded and analyzed with performance effectiveness evaluations based upon assigned group task outcome measures.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	James A. Carter, Ph.D.
ORGANIZATION:	Harvard – Beth Israel Deaconess Medical Center
PROJECT TITLE:	Designing a Smart Medical System for Psychosocial Support

Project Executive Summary

Project Aims

This project involves the prototyping and evaluation of a computer-based system to assist astronauts in preventing, assessing and managing psychological and social problems that can arise during long-duration space missions. In the prototype, we are addressing the problem areas of depression and conflict, drawing on the experience and expertise of veteran long-duration flyers as well as national experts in conflict resolution and depression treatment.

Specific aims of this project include:

1. Develop the architecture of a prototype Smart Medical System for Psychosocial Support
2. Develop a prototype computer-based system for self-assessment of depression
3. Develop a prototype computer-based system for the self-treatment of depression
4. Develop a prototype training module on conflict resolution
5. Evaluate the system for usability, acceptability, and perceived value with astronauts and experts in conflict and depression

Project Phases

The project involves three phases:

1. Conducting consultation interviews with former long-duration flyers from the International Space Station, Mir, and Skylab, as well as experts in our research team in conflict resolution and depression treatment.
2. Producing a proof-of-concept prototype of an interactive Smart Medical System for Psychosocial Support, including the basic interface and the frameworks of programs on depression and conflict management.
3. Evaluating the system's usability, acceptability and perceived value to astronauts.

Consultation interviews with veteran long-duration flyers.

Interviews with veteran long-duration flyers were an essential early step in developing the prototype because they are the best subject matter experts on the psychosocial experience of long-duration space flight. Interviews with these individuals provided value in the following ways:

1. Understanding the psychosocial environment
2. Identifying best practices for managing psychosocial problems on long-duration missions
3. Choosing and suggesting simulations to develop
4. Suggesting courses of action to take in simulations
5. Differences between Earth and space
6. Introducing the study to long-duration flyers to solicit their support and involvement
7. Identifying interviewees to be taped for use in the system

Participants were presented with five fictitious scenarios dealing with depression and conflict in space and asked for their opinions on:

- The best approaches (best practices) to managing each situation as a commander

- The best approaches (best practices) to managing each situation as a non-commander
- Undesirable actions to take in each situation
- Other options that might be available to deal with each problem
- The realism of each scenario
- Improvements that could be made to each scenario for training

Key Findings

Phase I has been completed, which involved securing IRB/CPHS approval from NASA-Johnson Space Center to interview space and ground crew personnel (a 6-month process), and conducting 11 interviews with veteran long-duration flyers: from the ISS (n=2), Mir (n=5), and Skylab (n=4) programs.

The interviewees provided a wide range of opinions, occasionally contradictory, regarding the best ways of managing the problems in the scenarios and pitfalls or missteps that might be made. Although all scenarios were judged realistic by the majority of participants, there were elements of some scenarios that did not ring true or could be improved. We gained insights into elements that should be added to the scenarios to enhance realism and will select one of these scenarios to develop into a full simulation. Finally, while one of our goals is to get concrete information on responses to scenarios, we are also obtaining a considerable amount of information about the “art” of dealing with problems on long-duration space flights that is hard to capture in a table, chart or graph but can be incorporated into the countermeasure through videotaped interviews with veteran flyers and simulations involving actors.

Across all scenarios, a general pattern of best practices emerged for *commanders* helping fellow crewmembers manage conflict and depression:

1. Discuss the problem with the affected crewmember
2. Try to help resolve the problem, with actions dependent on the nature of the problem
3. Consult with ground crew, with the affected crewmember’s consent. Contact the ground without crewmember’s consent if problem is serious or persistent
4. Involve the crewmember in problem-solving
5. Take an authoritative stance when needed, stressing that the mission is the crewmember’s top priority or giving ultimatums
6. Assess problems from multiple angles to find causes
7. Serve as a go-between for crewmember and ground

The following best practices pattern emerged for *non-commanders* helping fellow crewmembers to manage conflict and depression:

1. Discuss the problem with the affected crewmember
2. Refer the problem to the ground, with crewmember’s consent, if it is a serious or persistent problem
3. Identify the causes of the problem
4. Take action to fix the problem, such as sharing workload, or increasing communications with family, depending on the nature of the problem

Inappropriate actions, or pitfalls, that were most frequently indicated were:

1. Talking to the ground about another crewmember's problem without his or her consent—making the problem known outside of the space crew
2. Doing nothing about a problem
3. Blaming the crewmember for having the problem, considering him or her to be lazy, et. cetera
4. Not noticing problems or not appreciating their significance
5. Overreacting to problems

Impact of Findings

Findings from consultation interviews with long-duration flyers will directly inform the development of the Smart Medical System for Psychosocial Support by:

- Helping to identify appropriate and inappropriate responses to include in simulations to be developed for the System
- Helping to identify ways in which conflict and depression must be managed differently on long-duration space flights compared to on Earth
- Enabling us to integrate best practices from the perspectives of long-duration flyers and experts in depression and conflict management, ensuring that the program is based on empirically validated supported methods as well as being acceptable to astronauts
- Introducing veteran flyers to the project, to enlist their future support
- Identifying potential interviewees who may be videotaped on camera discussing best (and worst) practices to managing conflict and depression in space

Research Plan

We will attempt to complete this ambitious study on schedule. This year will involve the production of the prototype in with support from The Troupe Modern Media in Windham, New Hampshire, and evaluation of the prototype at NASA-Johnson Space Center.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	David F. Dinges, Ph.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Optical Computer Recognition of Behavioral Stress
END DATE:	02/29/2004

Project Executive Summary

The goal of this project is to develop and test an optically based computer recognition algorithm of the face to reliably detect the presence of stress during performance demands. As manned space flights of increasingly longer durations are being planned, there is evidence from U.S. and Russian space missions that astronauts and cosmonauts have experienced operational stressors that adversely affected subjective well-being, physiology, and performance capability. In order to provide countermeasures for stressor-induced impairments in astronauts, objective, unobtrusive measures of the presence of stress reactions are needed. This project seeks to achieve such a measure through collaboration between laboratories with expertise in the evaluation of behavioral and physiological responses under stressful and non-stressful conditions (Prof. D. Dinges, Department of Psychiatry, Unit for Experimental Psychiatry, The University of Pennsylvania), and in optical computer recognition of human subjects' facial expressions and gestures (Prof. D. Metaxas, Computer Sciences, Rutgers University).

Astronauts aboard extended-duration space missions will endure the harsh space environment and the effects of various stressors (e.g., microgravity, perceived risks, work requirements, habitability constraints, radiation, restricted communication with Earth) to a much greater degree than have been experienced previously. Maintaining individual neurobehavioral functioning of astronauts will be vital to assuring mission success. However, in order to provide countermeasures for stressor-induced, physical and functional impairments in astronauts, objective measures of the presence of heightened stress reactions are needed. The earlier that stress reactions (regardless of their operational, psychosocial, or neurobiological source) can be detected, the greater the probability that an appropriate countermeasure strategy can be implemented (e.g., rest, pharmacology, behavior).

In the absence of objective detection of developing stress reactions, it is unlikely that countermeasures or stress impairment of astronauts can be managed. Many techniques for monitoring stress reactivity in space flight are impractical (e.g., cortisol measurement), unreliable (e.g., self-report), or obtrusive. However, unobtrusive, continuous video monitoring of the human face during neurobehavioral tasks offers a potential solution to these problems. Consequently, this project provides the first scientific test of the use of optically based computer recognition of the face to unobtrusively and reliably detect the presence of stress during laboratory performance demands.

The computer-based optical recognition system builds on the research of Prof. Metaxas by utilizing automatic optical tracking of human subjects' anatomical and motoric changes in facial expressions during non-verbal performance tests. Video input to the system was provided from experiments performed in the laboratory of Prof. Dinges in which healthy adults (males and females of different ethnic backgrounds) were exposed to behavioral stressors to increase the likelihood of developing a sensitive algorithm. The aim of the protocol is to experimentally

establish whether an optical computer recognition algorithm based on facial expression can be developed that can objectively, independently and reliably discriminate when subjects are undergoing behavioral stressors, and whether a high degree of accurate categorization can be achieved for both male and female subjects for both younger (22-32 years) and older (33-45 years) subjects and for subjects of different ethnic backgrounds. Further, exploratory and heuristic analyses evaluate the effects of behavioral stressors on physiological responses of cortisol secretion and heart rate, on psychological responses of self-report ratings of stress and mood and on neurobehavioral performance responses; and explore the extent to which the magnitude of the stress response as assessed by these measures relates to the accuracy of the optically based computer recognition algorithm of the face.

A single-blind, repeated-measures controlled trial was used to achieve these aims and to provide the data required to test the hypothesis that an objective, unobtrusive, optically based computer recognition algorithm of the face can be developed to reliably detect the presence of high stress (and of low stress) during performance. A total of 60 healthy adults were studied in the Unit for Experimental Psychiatry laboratory (Dr. Dinges) during three sessions: I—screening session; II—training session for development of the optical computer recognition algorithm; and III—prospective test session of the predictive utility of the optical recognition algorithm to discriminate high versus low stressed states associated with behavioral stressors.

Stress reactions were tracked during both control (low stress) and high stress conditions in sessions II and III, by measurement of salivary cortisol, heart rate, subjective mood/stress responses, and neurobehavioral performance. Videos of subjects' faces in the low and high stress conditions of session II were used by the Vision Analysis and Simulation Technologies laboratory to develop a predictive optical algorithm that was tested blind to stressor level (i.e., high vs. low) in the behavioral stressor conditions of session III. The experiment is designed to test the hypothesis that an optical computer recognition algorithm can be used to discriminate when subjects are undergoing behavioral stressors as defined by established stress-related changes in cortisol secretion, heart rate, subjective reports, and performance.

Earth-Based Applications of Research Project

The study focuses on the ability of an unobtrusive, automated optical technology to detect psychological distress (and the need for countermeasures for it) during operational performance. The knowledge gained has the potential to identify an objective, unobtrusive, automated method for the recognition, monitoring, and management of the risks of neurobehavioral dysfunction due to work-related stress in spaceflight and in many Earth-based safety-sensitive occupations, such as transportation workers (e.g., truck drivers, train conductors, airline pilots); operators in safety-sensitive industries (e.g., power plant control rooms); and military personnel.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	David F. Dinges, Ph.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Optical Computer Recognition of Performance Under Stress

Project Executive Summary

Astronauts are required to perform mission-critical tasks at a high level of functional capability throughout space flight. There are a number of stressors that can compromise their ability to do so, making early objective detection of neurobehavioral problems in space flight a priority. The overarching goal is to develop an unobtrusive, automated optical technology to detect psychological distress (and the need for countermeasures for it) during operational performance. The primary aim is to determine whether optical computer recognition algorithms based on changes in facial expressions can discriminate behavioral stress induced by low versus high workload performance demands, and what influence facial edema, gender, age, ethnicity and alexithymia have on algorithm discriminability.

The proposed computer-based recognition system will build on promising results from current research on automatic optical tracking of human subjects' subtle anatomical and motoric changes in facial expressions during cognitive performance tasks. Eighty healthy adults (males and females of different ethnic backgrounds) will be exposed to laboratory simulations of varying degrees of workload-based behavioral stressors to develop a sensitive optical algorithm. The accuracy of the optical algorithm to detect stress in the face during performance will be evaluated both with and without facial edema, which occurs in microgravity. Stereo video image footage of the face gathered digitally at high resolution will be recorded during all low and high workload conditions, to train and test the algorithm.

The optically-based analysis of facial characteristics will consist of three different levels of representation that depend on the level of motion detail extracted. The first algorithm will consist of face detection, gross movement analysis and facial landmarks; the second will consist of constructing and representing a deformable face model; and the third will involve the computational detection of whether a subject is undergoing a stress reaction in response to a high workload. The results have the potential to identify an objective, unobtrusive, automated method for the recognition, monitoring and management of the risks of neurobehavioral dysfunction due to work-related stress in space flight and in many Earth-based safety-sensitive occupations.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Stephen M. Kosslyn, Ph.D.
ORGANIZATION:	Harvard College
PROJECT TITLE:	Quick Assessment of Basic Cognitive Function: Blood Pressure Cuffs for the Mind

Project Executive Summary

The goal of this project is to develop a set of brief performance tests (the MiniCog Quick Assessment Battery, or MQAB) and a method for administering them (the MiniCog application) on a handheld device or PDA. The performance tests have been selected from among standard tasks in psychological literature and tap basic cognitive abilities necessary for performing complex operations (such as extravehicular activity—EVA—or many other tasks that astronauts have to complete), or that have been shown to be impaired by the types of stressors that astronauts might expect to encounter (e.g., fatigue, anxiety, constant noise, demanding work shifts).

The tests are designed to be self-administered and the PDA program, MiniCog, is designed to provide immediate performance feedback. The specific tests implemented assess cognitive functioning in the domains of perceptual/motor control, attention (vigilance, divided attention, and filtering), verbal and spatial working memory, cognitive set switching, and verbal and spatial problem solving. The tool box developed here has three general applications: First, it will provide a practical means for assessing the effects of various stressors, environmental or internal, on key cognitive processes. For example, it could be used to assess the effects of fatigue, circadian rhythm disruption, mood disorders, exercise, drug use or diet on cognitive processing. Second, the test battery can be used to assess the effectiveness of "countermeasures," such as specific training to manage fatigue.

The tests can be administered before and after such training, which will provide a measure of how the training affects the basic information processes of interest. Finally, the tasks could be used by people "on the job" (e.g., astronauts in space) to inform themselves about the current state of their cognitive processes. For example, before EVA to repair an external part, an astronaut might find it useful to know how effectively she or he was reasoning logically and spatially. If one was not doing as well as usual, someone else might go, one might go later, or one might go but make a concerted effort to be very careful.

We have finished data collection for an 'individual differences' study using performance on MQAB as a predictor of performance on more complex and longer tasks. We are now analyzing data from that study. A study of the effects of fatigue on performance and the potentially ameliorating effect of caffeine is in the data collection phase. MQAB is also being used in several collaborations, including those with NSBRI team members Philip Lieberman and Judith Orasanu.

Earth-Based Applications of Research Project

The three general applications of this toolbox have "earth" benefits as well as "space" benefits: First, the toolbox will provide a practical means for assessing the effects of various stressors

(such as fatigue, exercise, drug use, or diet) on cognitive processing. While we expect some of those variables (e.g., fatigue, certain types of drugs) to have a significant impact on astronauts, they also affect people on earth (e.g., pilots on long flights suffer from irregular sleep schedules; a large percentage of the population takes psychoactive drugs; dieting and exercise are major concerns to many Americans).

Second, the tasks can be used to assess the effectiveness of "countermeasures," such as training or drugs to manage fatigue. Just as the same stressors may affect people on the ground as well as the astronauts, the same countermeasures may be useful for the earthbound population as well as those in spaceflight. Finally, the tasks could be used by people "on the job" (e.g. truck drivers) to inform themselves about the current state of their cognitive processes. For example, when truck drivers "weigh in" after many hours of travel, they can run through the battery of tests and make sure that they are still mentally alert. Depending on their scores, they may want to rest before going back on the road, or they may eat a meal or drink coffee, or engage in other activities to reduce the danger of driving. Other researchers will be able to use our test battery to assess cognitive performance quickly and under a variety of laboratory and field conditions; they may also use the MiniCog platform to develop their own psychological experiments.

Both our test battery and the MiniCog application have an advantage over standard task batteries and many typical psychological scripting programs in that the tests are brief and the method of administration is compact, portable, and fairly inexpensive. This could make MiniCog and MQAB practical in a wide range of settings where there are questions of neurocognitive capability.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Philip Lieberman, Ph.D.
ORGANIZATION:	Brown University
PROJECT TITLE:	Speech Monitoring Cognitive and Personality Alterations
END DATE:	06/30/2004

Project Executive Summary

Our long-term goal is a system that uses measures of speech to detect cognitive deficits, personality changes and emotional disturbances during prolonged space flight, allowing crews to invoke countermeasures before performance is severely impaired. The system will monitor conversation, deriving acoustic parameters via automated procedures, without requiring crewmembers to wear intrusive devices. It will protect privacy and mission security because it does not depend on the content of speech, only its acoustics.

Deep-space missions will expose crews to cosmic rays, which may damage brain structures including the basal ganglia and hippocampus. These subcortical structures are linked with cortical regions in circuits regulating motor control, cognition and personality. As models for crews suffering neurological damage, we use climbers ascending Mount Everest and patients with Parkinson's disease (PD). At altitudes above Everest Base Camp, the low oxygen content can damage the principal basal ganglia output structure, the globus pallidus. The hippocampus also is sensitive to hypoxia. The ascent of Everest also resembles long-term space flight in that a small group in close contact for an extended period must make critical decisions in stressful, life-threatening situations.

PD, which affects basal ganglia function, impairs motor control but also causes deficits in cognition and language comprehension more severe than those in Everest climbers. Climbers and PD patients offer complementary Earthbound space-analogs for milder and more extreme forms of the dysfunction astronauts may suffer on long-term missions. The rationale for using speech measures as cognitive indices is that the basal ganglia regulate sequences of speech motor acts as well as of cognitive operations. We can expect the corresponding circuits to show parallel declines in function due to hypoxia, PD or radiological damage. One index we focus on is voice-onset time (VOT). Stop consonants in word-initial position are produced by first closing the lips or placing the tongue against the roof of the mouth. The obstruction then is abruptly released, yielding a burst with distinct acoustic properties. Speakers must also adjust the larynx to produce a vowel after the burst. For voiced stops, like b, the time between burst and vowel, the VOT is less than ~30 msec, while for voiceless stops, like p, it is greater than ~50 msec. Hence, VOT production requires precise control of the sequence of oral and laryngeal actions.

PD patients show reduced differences between VOTs for voiced and voiceless stops. Such VOT convergence correlates with deficits in sentence comprehension. On Everest, we obtain measurements of VOT and other speech parameters, e.g., vowel duration, by using radios to record climbers reading words at different altitudes. We have measured cognition by various tests. Climbers carry picture booklets so that we can administer, by radio, sentence-picture matching tests of language comprehension. To measure cognitive flexibility, we give the Odd-Man-Out test (OMO) and, in 2004, the Wisconsin Card Sorting Task. These require subjects to

select stimuli using one of several possible criteria and then shift criteria following feedback. In 2003, we added the Mini-Cog test battery developed by Stephen Kosslyn's NSBRI group at Harvard. This battery, which tests cognitive capacities including verbal and spatial working memory, is implemented on Palm Pilot PDAs that climbers carry.

In 2004, a group of climbers also took an implicit contextual learning test that reflects hippocampal function. Results over successive expeditions bear out our hypotheses. At high altitudes, many climbers show VOT convergence and increased vowel duration. Crucially, these changes track impairment on cognitive tasks that depend on basal ganglia function. Greater VOT separation decreases were seen for larger increases in sentence comprehension response time (RT) with altitude. In 73% of cases, the presence (or absence) of reduced VOT separation signaled the presence (or absence) of deficits in comprehension RT or OMO performance. VOT separation had a similar 71% hit rate in discriminating impaired from unimpaired performance on Mini-Cog tests of working memory or vigilance. Vowel duration had a hit rate of 85% for sentence comprehension or OMO and was significantly correlated with verbal working memory RT.

Our speech metrics track severe cognitive deficits as well. One climber developed profound speech motor sequencing deficits and similarly extreme deficits in cognitive flexibility. Advised of his condition, he nonetheless proceeded to climb upwards, alone, through a storm; two days later he fell to his death. Preliminary analyses indicate that hypoxia impaired hippocampal function. Like amnesics, climbers at higher altitudes failed to show implicit contextual learning. Altitude did not affect performance on tasks that do not place demands on basal ganglia or hippocampal circuitry. Our PD studies support our hypotheses on the basal ganglia's role in understanding language.

By tracking eye movements as subjects do a sentence-picture matching task, we have shown that patients with high OMO error rates also exhibit impaired processing at points in sentences that require linguistic set-shifting. Our techniques may be used to monitor PD and have potential for detecting early memory loss in Alzheimer's disease, enabling interventions that could slow further decline. We are exploring the assessment of verbal apraxia in children, a disorder affecting linguistic and cognitive development that may result from hypoxia during birth. Our techniques have potential applications in general aviation, where hypoxia has led to disasters. In this project's next phase, we will apply speech analysis techniques to study task-induced stress in synergy with Kosslyn's group and David Dinges' NSBRI group at the University of Pennsylvania School of Medicine.

Earth-Based Applications of Research Project:

The techniques we are developing for unobtrusively monitoring cognitive status via automated measurement of speech parameters may have applications in general aviation. Systems based on these techniques could be used to monitor air crews for gradual effects of partial or slow failure of aircraft pressurization systems. The hypoxia resulting from such depressurization – which degrades cognitive function so that crew members not only are unable to perform their tasks but fail to notice their own impairment – has led to flight disasters in the past. Speech-monitoring techniques might also be useful in predicting the onset of Acute Mountain Sickness before it occurs in personnel deployed to high mountain regions. A speech-based system may also be useful in monitoring motor and cognitive dysfunction resulting from stress and sleep deprivation. Our project's techniques have already been used to assess the efficacy of new surgical procedures for the treatment of Parkinson's disease. They can be used to monitor disease state in

Parkinson's. They may also provide instruments that can detect memory loss in the early stages of Alzheimer's disease. Such early detection would permit clinicians to take maximal advantage of therapies, now under development, that can delay or even arrest further decline. In addition to Parkinson's disease, our techniques may have application to the diagnosis, assessment, and treatment of other human pathologies stemming from impaired dopaminergic basal ganglia function in neural circuits regulating speech production, cognition, and personality. These include not only neurodegenerative diseases but also the results of acute insult. For example, hypoxia during birth can lead to verbal apraxia in children – a syndrome where speech motor and orofacial motor control is degraded and which can result in cognitive and linguistic deficits.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Philip Lieberman, Ph.D.
ORGANIZATION:	Brown University
PROJECT TITLE:	Speech Monitoring of Cognitive Deficits and Stress

Project Executive Summary

The goal of this project is to derive and validate acoustic measures of speech that permit automatic and unobtrusive on-line monitoring of the effects of stress and neurological impairment on astronauts' ability to perform in extended deep-space missions.

Our project will integrate and validate ongoing NSBRI research projects aimed at systems that monitor an astronaut's ability to perform using (a) video recognition of facial markers of stress, (b) acoustic measures of stress and impaired cognition and (c) psychometric test procedures that permit the rapid assessment of cognitive ability. The project will establish a synergy between (1) a space-analog study involving climbers ascending Mount Everest, where life-threatening stress and neurologic impairment similar to that which may result from exposure to cosmic rays in deep-space missions occurs, and (2) the findings of laboratory-based studies of task-induced stress.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Judith M. Orasanu, Ph.D.
ORGANIZATION:	NASA Ames Research Center
PROJECT TITLE:	Distributed Team Decision Making in Exploration Missions
END DATE:	07/31/2004

Project Executive Summary

Since the beginning of human space flight, crucial incidents relating to the psychological, behavioral, and interpersonal aspects of crew performance have jeopardized crew safety and mission success in both U.S. and Russian programs (Kraft, 2003). While crewmembers are highly selected and technically skilled, "the history of space explorations has seen many instances of poor interpersonal relations and faulty decision making" (Committee on Space Biology and Medicine, NRC, 1998). Interpersonal relationships will be of particular concern as crewmembers become more diverse in terms of culture, gender and professional backgrounds (Connors, 1985; Kanas, 2001; Santy, 1993; Kraft, 2003).

Two major goals drive our research. The first is to understand the effects of *task and interpersonal stressors on team performance* in challenging distributed decision-making situations, and to identify effective strategies for coping with these stressors. The second objective is to *develop non-invasive technologies to detect low levels of stress* in individual team members to allow the introduction of countermeasures before team dynamics deteriorate to the point of threatening mission success. Findings will provide a basis for designing or revising crew selection, training practices, and procedures to monitor and support team performance.

A simulated task environment was developed to address our objectives. The task, a dynamic computer-based search and rescue mission set in Antarctica, provides an ideal environment for studying team interaction and problem solving because its underlying cognitive demands reflect those of many real-world team tasks. A four-person crew must develop plans, manage resources, and collaborate in order to find a lost party, accomplish its mission (repair a communications antenna), and cope with unexpected demands, such as clearing blocked terrain, rendering medical aid, and helping to repair broken-down vehicles. Graphical displays of the terrain and evolving problem scenarios are presented via linked computers; communication among team members is supported by e-mail and voice communication. Three crewmembers engage in the search using virtual snowcats, while a fourth remains at the base station to coordinate search activities. Two levels of difficulty were created for each of three scenarios by varying the diagnosticity of cues and time pressures. Team cohesiveness was manipulated by task goals: half of the teams were induced to work in a fully cooperative manner; the other half received instructions and feedback designed to induce conflict between team members.

All actions taken during the game and various outcome measures are automatically recorded by the game software. While participants work on the task, their physiological responses and facial expressions are recorded and monitored for signs of mental and emotional stress. Physiological responses, facial affect, and personality measures will be correlated with individual and team behaviors and overall task performance to build models of effective team composition and performance.

Year one of this project was devoted to establishing a distributed team decision making laboratory; the development and pre-testing of the simulated task environment, training materials

and six experimental scenarios; and the selection and pre-testing of a physiological monitoring and recording system.

In year two we selected a psychological assessment battery and ran a baseline experiment to assess the adequacy of the problem solving scenarios, the task manipulations, and the stress monitoring technologies during actual team interaction. Twelve teams each consisting of four U.S. males (at least second generation American) served as our baseline group. Team conflict varied between groups and task difficulty varied within teams.

Participants worked together over a period of four days in the laboratory. Day one consisted of training on the computer-based task and an introduction to the physiological monitoring devices. On days two, three and four, participants worked through six scenarios, one easy and one difficult scenario per day. Order was counterbalanced across and within days. Both individual and team performance measures were recorded for analysis. Personality measures were administered between tasks on Day two and three; state and group dynamic measures were taken at the end of each task day.

The manipulation of task difficulty was successful as players were generally more successful in completing tasks in "easy" scenarios than in "difficult" ones. The team conflict manipulation resulted in significant differences between groups, but in the opposite direction than expected. Scores earned by individual players in the Competitive condition were higher than those in the Cooperative condition were. Further analyses revealed that members of Competitive teams collaborated on tasks significantly more often than members of Cooperative teams. The superior performance of individuals in the Competitive condition may arise from several sources: differences in performance feedback provided in the two task conditions, in team strategies associated with each condition, or in cognitive abilities of the individuals randomly assigned to conditions. All three possibilities will be addressed in research during year three.

Team interaction and communication are analyzed from transcripts made from audio- and videotapes of the teams during planning and mission execution. Our initial review of team communication found both effective teamwork and negative affect. Effective task performance was found to depend on team collaboration: a coordinated search strategy, communication about mission-critical information, task assignment and prioritizing, and plan adaptation based on effective strategies were evident in successful teams. Team members established a sense of team cohesion and positive affect by treating each other with respect, by backing each other up, and by providing positive reinforcement for successful efforts and objective feedback on errors. Negative behaviors associated with team failure included players being withdrawn and passive, engaging in competitive behavior and deceit, and instigating conflict through negative feedback, criticism, and public embarrassment or humiliation of others. These behaviors will serve as the foundation for analysis of behaviors in culturally diverse teams in year three of the current grant and, once validated, as a basis for training in our follow-on proposal.

Physiological responses were monitored while players engaged in the search and rescue tasks. The time-coded output were filtered and cleaned prior to analysis; statistical analyses are still underway, but initial observations revealed physiological reactivity at points of task pressures.

Our plan for year three involves replicating the baseline study of year two with multicultural gender-mixed crews. The goal is to determine whether task and team stressors have similar effects on these diverse teams as on homogeneous male teams and whether physiological indicators will be similarly related to performance. Before conducting this study, we will run a second baseline study with a modified task context and performance feedback to ensure a more systematic manipulation of team conflict.

Given that the Antarctic search and rescue task context is inherently cooperative, the manipulation of team conflict by competitive instructions and individual rewards was less effective than anticipated. To induce conflict, thereby enabling the study of stress effects on crews, a new competitive game has been created. While structurally identical to the original search and rescue game, it is inherently competitive as it involves the search for ancient treasures stolen from a famous museum, hidden in Antarctica. Converting this inherently competitive task into a cooperative team task is relatively easy, in contrast to making the inherently cooperative search-and-rescue mission into a competitive one. A second change concerns the performance feedback in the Cooperative condition to include individual as well as team scores. These players will receive the same information regarding the success of their strategies as players in the Competitive condition. A third change involves the inclusion of a cognitive abilities test to control for possible inter-individual differences among study participants.

U.S. males (at least second-generation American) will participate in the new baseline study. Six teams will be assigned to each condition (Cooperative and Competitive). Physiological, biometric, personality, and group dynamic measures will be collected, as in the first baseline study.

The second study will involve culturally diverse and gender-mixed teams. Participants will be sought who are from countries involved in the International Space Station. College graduates (25-45 yrs old) whose English language skills are adequate to read instructions and communicate with team members will be invited to participate. Non-US participants must have been born in their native countries and have lived in the U.S. for less than two years. Based on the concept of cultural collectivism/individualism (Hofstede, 1984), we hypothesize that certain team compositions will lead to more effective performance than others will. Twenty-four teams (involving male and female players) will be included, half in the Cooperative condition and half in the Competitive condition. These groups will be compared with the U.S. male baseline group.

This project addresses Risk #18 under Human Behavior and Performance: *Human performance failure due to poor psychosocial adaptation*. This study will determine the extent to which task and interpersonal stressors affect performance on tasks that require team collaboration for successful performance. Moreover, the study will examine the impact of personality factors and cooperative behaviors, trust, and team cohesion as mitigating factors that can overcome the effects of situational stressors. Tools for assessing stress at both individual and team levels will serve as a foundation for introducing countermeasures.

Implications for future Earth-based research: The study provides integrated physiological, self-report, behavioral, and performance measures from teams working together over a period of time on a common task. Models based on replication with variations in team and task structures will significantly advance our theories of team performance under stress and provide a basis for training in diverse earth- and space-based environments.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	Judith M. Orasanu, Ph.D.
ORGANIZATION:	NASA Ames Research Center
PROJECT TITLE:	Enhancing Team Performance for Exploration Missions

Project Executive Summary

Since the beginning of human space flight, crucial incidents relating to the psychological, behavioral and interpersonal aspects of crew performance have jeopardized crew safety and mission success in both U.S. and Russian programs. Successful long-duration space missions will depend on the ability of crewmembers to collaborate effectively under highly stressful conditions. Interpersonal relationships will be of particular concern as crewmembers on long-duration space missions become more diverse in terms of culture, gender and professional backgrounds. Tensions are likely to result from miscommunications and misunderstandings based on differing cultural norms and expectations.

This research addresses Risk #18 in the NASA Critical Path Roadmap under Human Behavior and Performance: Human performance failure due to poor psychosocial adaptation. Critical questions to be addressed include: (a) what are fundamental behavioral and social stressors, (b) what factors contribute to the breakdown of team performance, and (c) what behaviors, personality traits and leadership styles inhibit performance?

This four-year project will examine (a) how team composition affects team effectiveness, affect and cohesion, (b) whether stress-identification strategies predict team performance across diverse crews, (c) whether there is an ideal group composition, and (d) the effectiveness of various training approaches to counteract team dysfunction.

Cultural diversity, gender and leadership in teams will be examined. Participants will be sought from countries that participate in the ISS. Effectiveness of interpersonal relationship training will be evaluated by comparing trained and untrained teams. During training, groups will develop team and interpersonal assessment tools. These tools will enable individuals to work effectively in future teams.

Results from this study will provide (a) technologies for predicting breakdown of team dynamics and performance; (b) guidelines for selecting, training, and assembling teams of astronauts to assure team compatibility and optimize performance; (c) tools for self assessment and team feedback; and (d) strategies for managing stress in multi-cultural and gender-mixed teams.

RESEARCH AREA:	Neurobehavioral and Psychosocial Factors
PRINCIPAL INVESTIGATOR:	JoAnna Wood, Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Individuals and Cultures in Social Isolation
END DATE:	02/29/2004

Project Executive Summary

This study was designed to examine the roles of personality, culture, and group influences on behavior, performance, and health outcomes in winter-over Antarctic stations. The planned two years of data collection are nearly complete, and preliminary analyses of data have begun. Three preliminary findings are encouraging.

- (1) The Helmreich PCI appears to tap dimensions of personality not measured with the 16PF, another recognized research tool.
- (2) Female station leaders perceive significantly less social support from fellow expeditioners than do female subordinates, male subordinates, or male leaders.
- (3) There appear to be both individual and group characteristics that influence interpersonal tensions.

These findings have significant implications for selection and training countermeasures.

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Team Leader: **Oman, C. M.** **MIT**

**Associate Team
Leader:** **Bloomberg, J. J.** **NASA JSC**

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RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Jacob J. Bloomberg, Ph.D.
ORGANIZATION:	NASA Johnson Space Center
PROJECT TITLE:	Understanding Full-Body Gaze Control During Locomotion
END DATE:	08/31/2004

Project Executive Summary

Control of locomotion requires precise interaction between several sensorimotor subsystems. Exposure to the microgravity environment of spaceflight leads to post-flight adaptive alterations in these multiple subsystems leading to postural and gait disturbances. Countermeasures designed to mitigate these post-flight gait alterations will need to be assessed with a new generation of functional tests that evaluate the interaction of various elements central to locomotor control.

Traditionally, gaze stabilization has been studied almost exclusively as a problem of eyehead-trunk coordination. However, coordination between the eye-head and trunk may not be the only mechanism aiding gaze stabilization particularly during locomotion. Therefore the first goal of this study was to determine how the multiple, interdependent, full-body sensorimotor subsystems aiding gaze stabilization during locomotion are functionally coordinated. The second goal was to use this information to develop new tests of locomotion function to be used to evaluate the efficacy of countermeasures.

To address the first goal, two experiments were performed. In the first study (Study 1) we investigated how alteration in gaze tasking changes full-body locomotor control strategies. Subjects (n=9) performed two discreet gaze stabilization tasks while walking at 6.4 km/hr on a motorized treadmill: 1) focusing on a central point target; 2) reading numeral characters; both presented at 2m in front at eye level. The second study (Study 2) investigated the potential of adaptive remodeling of the full-body gaze control systems following exposure to visual-vestibular conflict. Subjects (n=14) walked (6.4 km/h) on the treadmill before and after they were exposed to 0.5X minifying lenses worn for 30 minutes during self-generated sinusoidal vertical head rotations performed while seated. In both studies we measured: temporal parameters of gait, full body sagittal plane segmental kinematics of the head, trunk, thigh, shank and foot, accelerations along the vertical axis at the head and the shank, and the vertical forces acting on the support surface.

Results from Study 1 showed that while reading numeral characters as compared to the central point target: 1) compensatory head pitch movements increased, 2) the peak acceleration measured at the head was significantly reduced, 3) the knee joint total movement was on greater during the period from the heel strike event to the peak knee flexion event in stance phase of the gait cycle. Results from Study 2 indicate that following exposure to visual-vestibular conflict changes in full-body strategies were observed consistent with the requirement to aid gaze stabilization during locomotion.

Taken together, results from Studies 1 and 2 provide evidence that the full body contributes to gaze stabilization during locomotion, and that different functional elements are responsive to changes in visual task constraints and are subject to adaptive alterations following exposure to visual-vestibular conflict. These studies also successfully validate new integrated methodologies designed to assess locomotor function for countermeasure evaluation and validation.

To address the second goal of this study we developed a new test to measure dynamic visual acuity during treadmill walking. Astronauts returning from spaceflight experience reduced visual acuity during body motion of the kind experienced during walking due to alterations in gaze stability caused by neurovestibular adaptive changes. These changes in acuity have significant operational implications. The inability to see clearly during body motion can impair the ability to operate spacecraft, conduct EVAs and perform an emergency egress soon after landing following a long-duration spaceflight. Our newly developed dynamic visual acuity test allows us to measure changes both in static and dynamic visual acuity for both near (0.5 m) and far (4 m) visual target positions. This test was evaluated in both normal subjects and in patients with bilateral vestibular impairment. Results show a significant ability to reliably differentiate normal from clinical behavior.

We have used results obtained from this research to develop an in-flight measure of dynamic visual acuity. This test will measure static visual acuity while subjects stand on the ISS treadmill and dynamic visual acuity during treadmill walking. We are currently developing an integrated testing system using a computer driven microdisplay screen. This in-flight test will measure changes in static and dynamic visual acuity during the initial adaptation phase to spaceflight and during the full duration of the flight. This newly developed test will be performed on the ISS following delivery of the hardware via Shuttle flight ULF-2 in 2005.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Jacob J. Bloomberg, Ph.D.
ORGANIZATION:	NASA Johnson Space Center
PROJECT TITLE:	Development of a Gait Adaptability Training Program as a Countermeasure for Postflight Locomotor Dysfunction

Project Executive Summary

Following their return to Earth, crewmembers experience disturbances in their ability to walk and maintain postural stability due to neural adaptation to the microgravity conditions of space flight. These changes can pose risks to crew safety and mission objectives if nominal or emergency vehicle egress is required immediately following long-duration space flight. At present, no operational countermeasure is available to mitigate these risks by facilitating rapid sensorimotor re-adaptation to 1-G. Therefore, the goal of this project is to develop an inflight balance and gait training program that will facilitate recovery of locomotor function after long-duration space flight.

The proposed countermeasure is a gait training program based on the concept of adaptive generalization. During this type of training, the subject gains experience producing the appropriate adaptive motor behavior under a variety of sensory conditions and response constraints. As a result, the subject learns to solve a class of motor problems, rather than a specific motor solution to one problem, i.e., the subject learns response generalizability or the ability to "learn to learn" under a variety of environmental constraints.

The Gait Adaptability Training Program will be done simultaneously with nominal inflight treadmill exercise. By manipulating the sensory conditions of exercise, we will systematically and repeatedly challenge the balance control system during treadmill walking. This training regimen will enhance sensorimotor adaptability, facilitating re-adaptation to unit (Earth) and partial (Mars) gravity after long-duration space flight.

To develop the Gait Adaptability Training Program, we will conduct a ground-based study that will systematically investigate the training efficacy associated with exposing subjects to different combinations of sensory input during treadmill locomotion including alterations in visual flow, body loading, and support surface stability. We will also determine the optimal training schedule (distributed vs. massed) to maximize both the efficacy and efficiency of the training procedure. To determine the optimal training procedure and schedule, subjects will be evaluated upon completion of training on their ability to negotiate a complex obstacle course placed on an unstable support surface (medium-density foam) while experiencing a novel visuomotor transformation (20-degree displacing goggles).

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Charles M. Oman, Ph.D.
ORGANIZATION:	Massachusetts Institute of Technology
PROJECT TITLE:	Visual Orientation and Spatial Memory: Mechanisms and Countermeasures
END DATE:	02/29/2004

Project Executive Summary

When astronauts enter weightlessness, there is no sensation of falling, and normal simple head movements do not elicit disorientation and oscillopsia the way they often do in vestibular patients on Earth. However some astronauts experience persistent "inversion illusions", and most crewmembers occasionally experience startling "visual reorientation illusions" when they leave their seats and float sideways or upside down, or simply even watch another person doing this. The illusion results from a sudden realignment of the cognitive reference frame used for spatial orientation, and a disorienting change in the subjective identity of interior surfaces (e.g. ceilings seem like floors). As a result, crewmembers make reaching errors, and can even become momentarily lost within the vehicle. These illusions – which crewmembers often call "the downs" - are known to trigger space motion sickness. 0-G disorientation is among the primary biomedical risks of spaceflight as defined by NASA's Critical Path Roadmap. The goal of this multi-institutional, multi-investigator NSBRI neurovestibular research project is to better understand the process of visual orientation and spatial memory in 1-G and 0-G, and to develop countermeasures for these in-flight problems. Our specific aims are to study:

Human visual orientation. To better understand static and dynamic visual orientation illusions in 0-G by quantifying them in 1-G. To determine how visual frame, polarity, motion and gravireceptor cues influence the direction of the subjective vertical, the response of the oculomotor and motor control systems, stability of the visual world (oscillopsia), and how viewing one's own body, environmental brightness and color cues determine the subjective vertical. (L.Harris, I. Howard et al, York University)

Three-dimensional spatial memory and learning. To understand why astronauts have difficulty making spatial judgments between modules with different visual verticals, by quantifying how humans use visual cues in 1-G to establish "spatial frameworks" with in and between adjacent visual environments. To develop a computerized technique for teaching generic 3-D spatial orientation and memory skills. To investigate and evaluate ISS allocentric coordinate marking systems, and to develop a "virtual porthole" display so trainees can learn to visualize the spatial relationships of ISS modules and potential escape routes in three dimensions. (C. Oman, et al, MIT/W. Shebilske, et al, Wright State)

Neural coding of spatial orientation in an animal model. To define how the preferred direction of limbic system head direction cell depends on visual, vestibular, gravireceptive, proprioceptive and motoric cues in a rat animal model during three-dimensional locomotion. To understand how the vestibular system contributes to these head direction cell responses. Ultimately, to develop a neurophysiological understanding of visual reorientation illusions and spatial cognition in astronauts. (J. Taube, et al, Dartmouth).

Our research team is an interdisciplinary group of psychologists, physiologists, and engineers, with background in visual, vestibular and motor psychology and physiology, human and animal navigation and VR technology. We coordinate research through bimonthly teleconferences and

inter-laboratory visits, and actively collaborate with other colleagues at NSBRI and NASA Johnson Space Center. Facilities include unique tumbling rooms at York University, animal research facilities at Dartmouth, and several types of immersive virtual reality facilities at MIT, York, and Wright State University.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Charles M. Oman, Ph.D.
ORGANIZATION:	Massachusetts Institute of Technology
PROJECT TITLE:	Visual Orientation, Navigation and Spatial Memory Countermeasures

Project Executive Summary

The goal of this collaborative neurovestibular project is to develop four types of design, assessment, training and procedural countermeasures: a) Evidence-based spacecraft architecture and work-area design standards. b) Methods for quantitative assessment of inflight and postflight oscillopsia. c) Preflight visual orientation training techniques to reduce disorientation and improve inflight emergency egress. d) Teleoperation procedure and training improvements based on crewmember spatial skills.

The specific aims are:

1. To quantify how environmental geometric "frame" and object "polarity" cues determine human visual orientation, to support engineering of spacecraft and work areas;
2. To develop reliable means for quantifying head-movement-contingent oscillopsia;
3. To determine whether preflight virtual-reality techniques can improve astronaut 3-D spatial memory and navigation abilities by reducing "direction vertigo" and by teaching ISS configuration and emergency egress routes, and;
4. To improve astronaut teleoperation performance by taking into account the mental object rotation and "perspective taking" abilities of individuals while training and during operations.

In-flight spatial disorientation, spatial memory, navigation and teleoperation problems, and oscillopsia during re-entry and after landing have been identified as neurovestibular risks by Shuttle, Mir and ISS astronauts, NASA's Critical Path Roadmap, the Neurovestibular Adaptation Team Strategic Plan and a National Academy of Sciences committee report.

The project utilizes the unique virtual-reality research capabilities at York University and MIT. Six sets of experiments and extensions are proposed:

1. Measuring the effect of environmental geometry ("frame") cues using psychophysical techniques (York).
2. Assessing the influence of polarized objects on self-orientation perception using psychophysical judgments (York).
3. Assessing the extent and pattern of head-contingent oscillopsia and visual motion (York).
4. Effect of training-module orientation on inflight direction vertigo (MIT).
5. Influence of relative body orientation in preflight visual orientation and egress training (MIT).
6. Correlation of spatial abilities with simulated space station remote-manipulator training performance (MIT).

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Lakshmi Putcha, Ph.D.
ORGANIZATION:	NASA Johnson Space Center
PROJECT TITLE:	Pharmacotherapeutics of Intranasal Scopolamine

Project Executive Summary

Space Motion Sickness (SMS) is commonly experienced by astronauts and often requires treatment with medications during the early flight days of a mission. SMS medications typically cause performance and cognitive function deficits. Bioavailability of oral SMS medications is often low and highly variable; additionally, physiological changes in a microgravity environment exacerbate variability and decrease bioavailability. Intranasal administration of scopolamine achieves higher and more reliable bioavailability than from an equivalent oral dose. However, efficacy and pharmacodynamics of intranasal scopolamine have not been characterized.

This project addresses Critical Path Roadmap Risk 45, Critical Question 11.19 (Priority 1). We hypothesize that intranasal scopolamine will (a) be efficacious and reliable, (b) be effective at lower doses than are used orally, and (c) minimize the incidence and intensity of neurocognitive side effects. The research objectives are: 1) establish efficacy and pharmacodynamics of intranasal scopolamine for SMS suppression and side effects, and 2) estimate bioavailability of intranasal scopolamine in a ground-based microgravity analog – anti-orthostatic bed rest.

Overall experimental design consists of three separate sessions.

Session One: Pharmacodynamics of intranasal scopolamine will be determined in 12 subjects with four escalating dose levels between 0.1 and 0.4 mg.

Session Two: Eighteen healthy subjects will participate in a dose-ranging study protocol to compare the effectiveness of 0.2 (low dose) and 0.4 mg (high dose) intranasal scopolamine to suppress motion sickness induced by off-axis vertical rotation.

Session Three: Bioavailability of two doses (0.2 and 0.4 mg) of intranasal scopolamine will be examined during ambulation and during anti-orthostatic bed rest in 12 subjects.

Serial blood and saliva samples and void-by-void urine samples will be collected for 24 hours post drug administration, a PALM-based sleepiness score and performance test battery (ARES) will be completed. Samples and data will be analyzed using established methods. Results of this study are expected to provide an easily administered, rapid-acting and effective treatment for SMS.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Millard F. Reschke, Ph.D.
ORGANIZATION:	NASA Johnson Space Center
PROJECT TITLE:	Modification of Eccentric Gaze-Holding

Project Executive Summary

Clear vision is a prerequisite for reliable performance of motor tasks. Space flight confronts the crewmember with a stimulus rearrangement that requires adaptation to function effectively with the new requirements of altered spatial orientation and motor coordination. Adaptation and motor learning driven by the effects of cerebellar disorders may share some of the same demands that face our astronauts.

One measure of spatial localization shared by the astronauts and those suffering from cerebellar disorders that is easily quantified and for which a neurobiological substrate has been identified, is the control of the angle of gaze (the "line of sight"). The disturbances of gaze control that have been documented to occur in astronauts, both in-flight and post-flight, can be directly related to changes in the extrinsic gravitational environment and intrinsic proprioceptive mechanisms, thus lending themselves to description by mathematical models. The basic models can be formulated using normal, non-astronaut test subjects and subsequently extended using centrifugation techniques to alter the gravitational and proprioceptive environment of these subjects. Further tests and extensions of the models can be made by studying abnormalities of gaze control in patients with cerebellar disease. Finally, tests of astronaut subjects during and after exposure to space flight, in association with the corresponding sensory-motor adaptations, will allow us to evaluate and extend our developed understanding of adaptation in the control of eccentric gaze-holding.

The specific aims of this study are:

1. To investigate the mechanisms of gaze-holding in normal, non-astronaut subjects, with the head held in various orientations with respect to gravity and the head held in various orientations relative to both gravity and the trunk. This will involve characterizing the *time constant of centripetal gaze drift*, the rate in which the eyes naturally drift back toward the null position following an eccentric eye movement.
2. To investigate the mechanisms that adaptively compensate for gaze-holding failure, especially the "rebound nystagmus" phenomenon, which decreases the rate of centripetal drift of the eyes. We will study the time course of rebound nystagmus in normal, non-astronaut subjects.
3. To investigate the stimulus rearrangement and adaptation resulting from exposure to gravito-inertial environments *greater* than 1 G using prolonged exposure to centrifugation.
4. To study mechanisms that adaptively compensate for gaze-holding failure in patients with vestibular cerebellar disease who show impaired gaze-holding ability. We will compare gaze-holding defects and rebound nystagmus in patients with that obtained in our normal subjects.
5. To compare the gaze-holding abilities of astronaut subjects prior to, during, and immediately following space flight with specific predictions made as a consequence of the ground-based research. Tests similar to those performed upon normal, non-astronaut subjects will be conducted to quantify changes in the time constant of centripetal drift of the eyes in relation to changes in the gaze-holding induced as a result of the stimulus rearrangement of space flight.

6. To measure the stability of gaze, during all phases of flight, with the eye at the central position in astronauts to investigate the occurrence of saccadic intrusions known as "square wave jerks" (SWJ), and to relate SWJ mechanisms common to the failure of gaze-holding.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Mark J. Shelhamer, Sc.D.
ORGANIZATION:	Johns Hopkins University School of Medicine
PROJECT TITLE:	Context-Specificity and Other Approaches to Neurovestibular Adaptation
END DATE:	02/29/2004

Project Executive Summary

There are several operational issues involved with altered human performance during and immediately after space flight. These issues have implications for human safety and effectiveness. Our planned experiments are designed to give us the information needed to develop and assess appropriate countermeasures (pre-flight or in-flight activities) for the vestibular deconditioning that occurs during flight (and often persists upon return to a planetary environment). Whenever g-transitions occur, there is a very real possibility of disruptions in perceptual and sensorimotor processing and reflex calibrations. These can have serious consequences in a dynamic environment such as shuttle re-entry or Mars landing.

We propose context-specific adaptation (CSA) as a countermeasure to some of the deleterious neurovestibular effects of space flight. By CSA we mean the ability of an organism to 1) maintain two different adapted states for a response (such as two different saccade gains), 2) have each state associated with a specific context (such as g level), and 3) switch between the adapted states immediately upon a change in context (i.e., without de-adaptation and re-adaptation upon each transition). This phenomenon can be useful during phases of space flight that require transitions between different g environments (e.g., in and out of artificial gravity, from orbital flight to planetary landing). A related theme is the determination of effective adaptation procedures and effective context cues. The role of the cerebellum, and its possible disruption during flight, is another central issue, as is transfer of adaptation between motor systems.

Outline of sub-projects in this proposal

Our project consists of an integrated set of experiments that have as their overall goal the design of a spaceflight countermeasure based on forms of vestibular adaptation. Briefly, the experiments include three main investigations at Johns Hopkins: 1) studies on the effects of torsional misalignment, and the use of saccade adaptation and cyclovergence adaptation as countermeasures (Shelhamer/Zee, aims 1-3), 2) studies on the relationship between the LVOR and smooth pursuit and the role of the cerebellum on adaptation of these responses (Zee/Minor/Shelhamer, aims 4-6), and 3) a study on context cues in the human LVOR (Shelhamer, aim 9). Another set of experiments will be conducted at Washington University (St. Louis) to study how CSA might transfer between eye movements and limb movements (Angelaki/Snyder, aim 7), and experiments at the University of Mississippi Medical Center will investigate adaptation of the LVOR with transient accelerations (Zhou, aim 8).

General outline of the progress report

The research progress described in this report represents something of a major redirection of effort from the previous reporting period, as some projects wind down (Aims 7 and 8) and others focus on new findings (e.g., Aims 1-3 focusing on skew). We also take advantage of related ongoing work and its applicability to some of the neurovestibular problems of space flight (the LVOR stimulated by small rapid translations, vertical saccade asymmetries).

Specific Aims (as originally planned)

1. To determine if static torsional eye position (induced by a visual display or by parabolic flight) can be used as a context cue for the adaptation of saccade metrics. Previous work implies that torsional changes in flight may affect saccades and other spatially-oriented behaviors. We will attempt to demonstrate that saccades can be made veridical in two different torsional states.
2. To see if CSA can be more readily acquired by allowing consolidation of adaptation to take place before changing contexts. We will allow for consolidation of each adapted state to occur by inserting a rest interval between the two context states during the CSA procedure.
3. To develop cyclovergence adaptation as a countermeasure to torsional offsets during changes in gravity. A visual stimulus can be used to induce torsional misalignment (cyclovergence). We will design an effective cyclotorsion adaptation stimulus in lab experiments, and use it to maintain the usual (1g-based) torsional alignment during parabolic flight, and see if otherwise inappropriate responses (saccades) in flight are evoked correctly if torsion is "corrected" to its normal (1g) state.
4. To compare horizontal and vertical pursuit and LVOR deficits over a wide range of frequencies, in cerebellar patients and in monkeys with vestibulocerebellar lesions.
5. To study in normal humans, and in monkeys before and after vestibulocerebellar lesions, pursuit and LVOR adaptation and their transfer over a wide range of frequencies.
6. To study in normal humans, and in monkeys before and after vestibulocerebellar lesions, CSA of the LVOR and in particular the ability to use pursuit stimuli with different g cues as a stimulus for learning multiple LVOR gains as a function of the g state.
7. To determine if CSA learned in one behavior (eye movements) will transfer to a different behavior (arm movements) in rhesus monkeys. We will use static head tilt as a context cue to adapt either the horizontal AVOR or horizontal saccades. Then we will investigate whether this context-specific adaptation is also present in memory-guided saccades and reaching. Experiments will be performed in intact animals and in animals with cerebellar lesions.
8. To use the transient linear vestibulo-ocular reflex (LVOR) to study context-specific otolith-ocular adaptation in human subjects. Our goal is to find the most effective procedure for adaptation of the transient LVOR, in anticipation of its possible use as part of a space flight countermeasure. (a) Systematically characterize task-specific LVOR adaptation in human subjects. (b) Identify the most effective training protocols to induce context-specific adaptation in human subjects. (c) Test for the ability of visual cues to substitute for vestibular cues in context-specific LVOR adaptation in human subjects.
9. To study CSA in the naso-occipital LVOR as for the inter-aural LVOR, and to determine what context cues are effective in each case.

Key findings and their impact

1. Two negative findings are of interest. First, we found that there is not a noticeable vertical error in horizontal saccades in parabolic flight. We thought that there might be, based on separate findings of disconjugate torsion in altered g levels, and saccade errors with the eyes deviated torsionally. The fact that we did not find such an error in the altered g levels of parabolic flight suggests that we can rule out this aspect of saccade accuracy as a confounding sensorimotor issue in space flight. The other negative finding is that asymmetries in vertical saccades (gain and latency) do not appear to be solely, or even predominantly, gravity-related.

Again, this suggests that vertical saccades are likely not adversely affected during flight, at least as a direct consequence of altered g level.

2. A very significant and unexpected finding is the presence of vertical ocular misalignment (skew) during the altered g levels of parabolic flight. This was found in the course of the investigations on saccade error described above. It was first noticed when subjects reported that they saw a single small target light split into two (diplopia), and that they could not fuse the two images, especially in the 1.8 g phase of flight. This phenomenon may be another consequence (as disconjugate torsion likely is) of otolith asymmetry, and has clear implications for piloting and other tasks during g transitions.

3. Our various findings on the properties and adaptability of the linear VOR (LVOR) have increased our base of knowledge of this fundamental response. We hope to use this paradigm as a test of otolith and cerebellar function in the future. In particular we feel that it can be an important part of a standard pre-flight/post-flight vestibular test battery for flight crews.

Research plan

The overall plan for year three is substantially changed from that originally outlined. In particular, we intend to follow up intensively on our new findings on vertical skew (including its extent, adaptation, and visual consequences), and pursue the development of the LVOR as a test of otolith and cerebellar function.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Conrad C. Wall III, Ph.D.
ORGANIZATION:	Harvard – Massachusetts Eye and Ear Infirmary
PROJECT TITLE:	Advanced Techniques to Assess and Counter Gait Ataxia
END DATE:	08/31/2004

Project Executive Summary

The overall goals of this project are to develop “countermeasure assessment criteria” to evaluate recovery from disturbances, and during turning, circular walking and ascending and descending stairs. We also consider countermeasures using a balance prosthesis and dynamic exercises designed to challenge and increase subjects’ balance. We will determine the sensitivity of the countermeasure assessment criteria in evaluating effects the prosthesis and the exercises on postural stability and locomotion. Using human subjects, the specific aims of this project are to:

1. Study body and head movements during precise perturbations of gait during continuous straight locomotion.
2. Study body, head and eye movements during continuous straight or circular locomotion on a circular treadmill.
3. Study body, head and eye movements during ascending and descending a staircase.
4. Study body, head and eye movements during standing, linear walking and treadmill walking with a balance prosthesis designed as a countermeasure for vestibular adaptation.
5. Study the effect of dynamic balance exercises for vestibulopathic subjects upon their ability to stand quietly and to recover from mild perturbations.

Key findings of the project

We have developed an experimental protocol that introduces a calibrated disturbance to the foot during the support phase of normal locomotion. This provides a means for the objective quantification of locomotor response dynamics that are known to be altered in astronauts upon return from exposure to microgravity but for which no current test exists. Returning astronauts whose orientation mechanism has been distorted and patients having balance disorders (vestibulopathies) that may well affect their orientation mechanism was expected to have different recovery trajectories than healthy normals. This is has now been demonstrated for vestibulopathic subjects. A simplified version of our research device is now being developed for use in evaluating the functional mobility of astronauts by scientists at the Johnson Space Center.

One of our working hypothesis was that profound impairments of posture, gaze and locomotion stability are caused by alterations in compensatory and orientation mechanisms that are generated in the central vestibular system from motion inputs. During exposure to altered gravity, the motion inputs from the otolith organs are “distorted” compared to the on-earth conditions. These distortions, in turn, cause both inappropriate body head and eye movements and an altered sense of orientation, which degrades stability during locomotion. We compared motions of the body during walking along a straight line with body motions while walking along a curved path. In the latter condition subjects accelerate in toward the direction of the curve, which introduces an inertial component which may or may not effect measures of their body orientation in space. Our results show that compensatory eye, head and body movements stabilize gaze during straight walking, while orienting mechanisms direct the eyes, head and body to tilts of the resultant of gravitational and centripetal acceleration in space during turning. This finding in normal subjects can now be compared to subjects with known impairments in their balance system or to returning astronauts to determine whether or not such individuals can successfully align parts of their bodies in an appropriate way while turning.

We have developed the simplified precursor to a balance aid. It uses body mounted motion sensors to estimate the tilt of the subject. This estimated tilt is coded and fed back to the subject using an array of small, non-invasive tactile vibrators mounted on the skin. The application of vibrotactile display of body tilt demonstrates for the first time (to our knowledge) that tilt estimates derived from body-mounted motion-sensing instruments can actually be used to reduce sway in subjects who have documented deficits in their balance (vestibular) function. The single most important finding was that subjects who repeatedly fell under challenging balance conditions were able to stand with the use of this aid.

Impact of these findings on the hypotheses or requirements (technology), objectives and specific aims of the original proposal and the proposed research plan for the coming year

These findings indicate that the overall objectives of the project are being met. The research plan for the coming year will remain as originally planned.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Scott J. Wood, Ph.D.
ORGANIZATION:	NASA JSC
PROJECT TITLE:	Sensorimotor Adaptation Following Exposure to Ambiguous Inertial Motion Cues

Project Executive Summary

The central nervous system must resolve the ambiguity of inertial motion sensory cues in order to derive an accurate representation of spatial orientation. Previous studies suggest that both frequency segregation and multi-sensory integration are complementary strategies used for discriminating linear accelerations arising from tilt and translation head motion. Adaptive changes during space flight in how inertial cues from the otolith system are integrated with other sensory information lead to perceptual and postural disturbances upon return to Earth's gravity.

We hypothesize that multi-sensory integration will be adaptively optimized in altered gravity environments based on the dynamics of other sensory information available, with greater changes in otolith-mediated responses in the mid-frequency range where there is a crossover of tilt and translation otolith-mediated responses. The first phase of our experiments is designed to elucidate physiological mechanisms for re-entry disturbances and to develop a ground-based adaptation model for evaluating adverse operational implications of tilt-translation adaptation.

The first specific aim of this proposal will be to examine the effects of stimulus frequency and different patterns of inertial sensory cues on adaptive changes in eye movements and motion perception during combined tilt and translation motion profiles. For our second specific aim, we will employ a closed-loop nulling task in which subjects will be tasked to use a joystick to null out tilt motion disturbances with or without concomitant translation motion. Our final specific aim is to evaluate how a tactile prosthesis can be used to improve control performance.

The results of this study will contribute to refining the ability of the tactile prosthesis to improve spatial orientation and navigation and serve as a countermeasure for tilt-translation disturbances during and following G-level changes.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Laurence R. Young, Sc.D.
ORGANIZATION:	Massachusetts Institute of Technology
PROJECT TITLE:	Neurovestibular Aspects of Artificial Gravity Created by Short-Radius Centrifugation
END DATE:	02/29/2004

Project Executive Summary

This project seeks to provide answers for the effects of artificial gravity on human orientation and eye, head, and limb movements, and the pros and cons of various types of artificial gravity as countermeasures against the effects of micro-gravity on neurovestibular function.

It was thought until recently that AG could be implemented only by using large-radius rotating spacecraft. We have further challenged the assumption that 4-6 rpm is a natural barrier beyond which adaptation becomes unattainable. Fast-spinning small rotational devices are a practical alternative. An integrated set of studies carried out at 3 universities treats the practical issue of adapting subjects to AG rapidly and with minimal motion sickness (MS) or motor disturbance.

We have shown that adaptation to high rotation rates (23rpm) appears in physiological measures and in subjective responses (MS scores, illusory tilt and cross axis vestibular ocular reflex) when confining head movements to one quadrant in a single plane (yaw head turns while supine). By the end of the second exposure a significant reduction in all three measures to about 1/3 of initial values is found and the adaptation is maintained over a five-day rest period. Further, adaptation generalizes quickly to the same head turns made while reversing the direction of centrifuge rotation. Likewise, pilot data suggest that adaptation to yaw head turns made in the right quadrant generalizes to the left quadrant within a few minutes. The changes due to adaptation are retained between exposures and are partially maintained over a five-day rest period.

The three different measures adapt to Coriolis stimulation in different ways, and an augmented visual-vestibular conflict did not facilitate adaptation uniformly.

Artificial Gravity (AG), especially when combined with exercise, is an attractive antidote to some of the deleterious effects of long-duration space flight. Since practical constraints forbid large-radius spinning spacecraft or tethers, emphasis has shifted to short-radius centrifuges (SRC). This entails intermittent exposure to high rotation rates and the likelihood of motion sickness when out-of-plane head movements are made.

The concern that high-speed rotation would be unacceptable for reasons of motion sickness was addressed and essentially resolved. The 21 published articles from this grant include documented, peer-reviewed papers explaining just how adaptation to AG is achieved and maintained.

Earth-Based Applications of Research Project

Head movements in a moving or rotating environment - such as in boats, airplanes, and automobiles - are often provocative. The ability to control susceptibility to motion sickness by controlling the central time constant of the vestibular system is a major advance and has broad application on Earth.

RESEARCH AREA:	Neurovestibular Adaptation
PRINCIPAL INVESTIGATOR:	Laurence R. Young, Sc.D.
ORGANIZATION:	Massachusetts Institute of Technology
PROJECT TITLE:	Neurovestibular Aspects of Short-Radius Artificial Gravity: Toward a Comprehensive Countermeasure

Project Executive Summary

Artificial gravity (AG), produced by centrifugal force on a rotating spacecraft or an on-board centrifuge, is a promising general countermeasure to the debilitating effects of weightlessness. However, high-speed rotation above 180 degrees/second is necessary to produce 1-G or more on a short radius (1.5-3 meter) centrifuge. Any astronaut head movement not parallel to the plane of rotation can induce strong cross-coupled spatial disorientation, motion sickness, postural disturbance and non-stabilizing compensatory eye movements. This project addresses the issues of adaptation to Coriolis forces and cross-coupled accelerations in accordance with the artificial gravity aim of the NSBRI's Neurovestibular Adaptation Team. The goal of this project is to understand the side effects caused by cross-coupled stimulation that produce motion sickness and could interfere with cognitive and motor function. A further goal is to develop efficient means of adapting astronauts safely to repeated transitions into and out of AG without excessive motion sickness. Basic understanding of the roles played by vestibular and other sensors in adaptation to unusual environments, and the associated disorientation and motion sickness, will contribute to astronaut comfort and safety in all phases of flight.

Fundamental studies of the process of sensory-motor adaptation and practical means of controlling motions sickness and sway during rotation are combined in our five Specific Aims. 1) Acquisition, Generalization and Retention of Adaptation (MIT). 2) Cognitive Influences on Adaptation, and Effects of AG on Human Performance (JSC and MIT). 3) Spatial Orientation as Influenced by AG (MIT and Brandeis). 4) Adaptation of Postural Sway during AG (Brandeis). 5) Effectiveness of Baclofen in Controlling Motion Sickness by Shortening the Vestibulo-Ocular Reflex Time Constant (Mount Sinai School of Medicine).

Human rotators spinning about an Earth-vertical axis provide the stimuli for each investigation: a rotating bed at MIT, an on-axis chair at Mt. Sinai, a 3-meter radius rotating room at Brandeis, and a 1.5-meter centrifuge at JSC. Measurements are made of compensatory eye movements, dynamic visual acuity, reading comprehension, illusory body motions, subjective motion sickness and postural sway.

**NSBRI RESEARCH PROGRAM
NUTRITION, PHYSICAL FITNESS AND REHABILITATION**

Team Leader:	Lupton, J. R.	Texas A&M		
Associate Team Leader:	Caiozzo, V. J.	UC, Irvine		
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Smith, S. M.	CO-I	NASA JSC		
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RESEARCH AREA:	Nutrition, Physical Fitness & Rehabilitation
PRINCIPAL INVESTIGATOR:	Marco E. Cabrera, Ph.D.
ORGANIZATION:	Case Western Reserve University
PROJECT TITLE:	Metabolic Adaptations of Skeletal Muscle to Training/Detraining: A Systems Model
END DATE:	08/31/2004

Project Executive Summary

Space travel (*detraining*) has detrimental effects on skeletal muscle structure, metabolism, and function, including reductions in muscle size, strength, and endurance. Exercise (*training*) in space can counteract some of these deleterious effects. Indeed, experimental studies are still being conducted to determine both the cause of muscle deterioration and the exercise training programs needed to counteract the detrimental effects of long-duration space travel on muscle function. In addition to obtaining relevant metabolic data from space and ground-based studies, physiologically-based computational models of human function are needed to integrate cellular to whole-body data and to provide a framework for quantitative understanding of the skeletal muscle metabolic responses to exercise in the trained and detrained states.

The specific aims of this project are:

1. To identify the metabolic adaptations to training and detraining in order to develop databases containing (a) information on the structural, metabolic, and functional adaptations of skeletal muscle to microgravity and exercise training and (b) the underlying biochemical mechanisms mediating these adaptations.
2. To develop mathematical models of intermediary metabolism in skeletal muscle that account for the effects of training and detraining.
3. To investigate the relative significance of model parameters affected by training or detraining on work capacity and efficiency.
4. To simulate the effects on skeletal muscle intermediary metabolism and energetics of space flight and exercise in space, to quantitatively test the hypotheses that after a period of space travel or exercise training, the observed changes in the rates of carbohydrate and fatty acid oxidation in skeletal muscle are a result of (a) a partial conversion of slow-twitch to fast-twitch fibers and (b) alterations in glycolytic and oxidative enzymes.

During the second year of the project we continued the development and implementation of a computational model of skeletal muscle metabolism that integrates cellular, tissue, and whole body data and that incorporates specific parameters which have been identified as playing a major role in the responses to training and detraining such as muscle mass and enzyme activities. Computer simulations of responses to moderate exercise were performed on three muscle models representing different states: (a) normal sedentary subject, (b) trained subject, and (c) detrained subject. Then, we contrasted the exercise responses resulting from the model of a "trained muscle" to those from the model of a "detrained muscle."

We also continued collaborating with other NSBRI investigators in the development of:

- a) A comprehensive model of the human body and its responses to exercise, in collaboration with Dr. Martin Kushmerick (Muscle Alterations Team) and Dr. James Coolahan (Cardiovascular Alterations Team), and
- b) Methods to evaluate the effectiveness of exercise training programs in space, in collaboration with Dr. Babs Soller (Smart Medical Systems Team).

RESEARCH AREA:	Nutrition, Physical Fitness & Rehabilitation
PRINCIPAL INVESTIGATOR:	Joanne R. Lupton, Ph.D.
ORGANIZATION:	Texas A&M University
PROJECT TITLE:	Nutritional Countermeasures to Radiation Exposure
END DATE:	09/30/2003

Project Executive Summary

1. Original Aims. The overall goal of this research program is to develop nutrition countermeasures to radiation-induced colon tumorigenesis, using male Sprague Dawley rats as a model system. Superimposed on the background of irradiation with Fe-ions or no irradiation is the injection of a known colon specific carcinogen, azoxymethane (AOM) in order to simulate the potential exposure to environmental contaminants. The diet interventions to be tested are combinations of a lipid component (fish oil vs corn oil) and a fiber component (pectin vs cellulose). At the end of the three year period we will know: (1) if radiation exposure synergistically enhances colon tumor induction by AOM; (2) which diet combination(s) are protective against colon cancer and if this effect is due to less DNA damage, greater removal of DNA-adducted cells by apoptosis or greater repair of DNA-adducted cells; (3) if short term studies (e.g. initiation or aberrant crypt formation) are predictive of later tumor development; and (4) if noninvasive technology can be used to detect specific mRNAs that are predictive for radiation exposure and/or response to that exposure, which would have later application to humans.

2. Key Findings. We have discovered that prior exposure to radiation before exposure to a chemical carcinogen increased the severity of preneoplastic lesions formed during colon tumorigenesis. Expression of genes associated with response to carcinogen exposure, as well as control of cell cycle kinetics is influenced by radiation exposure, above and beyond that observed with the chemical carcinogen. The dietary manipulations demonstrate that fermentable fiber and n-3 fatty acids are able to reduce the formation of preneoplastic lesions of colon cancer. Radiation treatment, combined with carcinogen exposure, also induces cancers in other tissue sites. Tumors in breast epithelia and in bone have not been seen with the chemical carcinogen alone. Development of these tumors necessitated the earlier termination of rats at the tumor time point.

3. Impact of Findings on Project Goals. The initial findings from our experiment indicate: 1) radiation exposure prior to a chemical carcinogen increases the risk of colon cancer development, and 2) diet is able to reduce the number and severity of preneoplastic lesions that lead to colon cancer. Therefore, diet may serve as a viable countermeasure to help maintain astronaut health.

4. Proposed Research Plan for the Coming Year. Analysis of samples collected from the last half of the rats treated only with the carcinogen will be completed by August 30, 2004.

RESEARCH AREA:	Nutrition, Physical Fitness and Rehabilitation
PRINCIPAL INVESTIGATOR:	Joanne R. Lupton, Ph.D.
ORGANIZATION:	Texas A&M University
PROJECT TITLE:	Nutritional Countermeasures to Radiation-Enhanced Colon Cancer

Project Executive Summary

One of the most serious impediments to long-duration space flight is radiation-enhanced tumorigenesis, and colon cancer is a likely candidate, being the second leading cause of death from cancer in the United States. It is also the cancer most responsive to diet intervention, suggesting the potential of diet as a countermeasure. The goal of our initial NSBRI grant was to determine if radiation exposure prior to a colon carcinogen enhances colon cancer development and if diet can serve as a countermeasure. The renewal will use fecal material and colonic mucosa (which have been collected and appropriately stored) from the same rats as the parent grant.

The primary goal of this continuation grant is to develop a set of colonocyte gene expression profiles that accurately characterize the radiation-enhancing effect on colon carcinogenesis and the chemoprotective effect of dietary countermeasures. The secondary goal is to compare gene expression profiles developed from exfoliated cells to those from colonic mucosal cells to determine how well they reflect *in vivo* events. This information is important for our short-term future goal of using feces from humans as a noninvasive method to monitor changes in gene expression over time. Our long-term future goal is to have sets of gene expression profiles that can be used to monitor astronauts before, during and after space flight to detect exposure and response to a radiation event so that appropriate intervention strategies (e.g. dietary countermeasures-Nutrition Team Goal) can be implemented. Data generated from this project will support the NASA/NSBRI goals of 1) gaining greater understanding of the mechanisms involved in the synergy of radiation and other environmental insults on carcinogenesis; 2) developing a system to monitor the need for countermeasure administration during or after flight; and 3) development of countermeasures that can be applied before, during and after flight.

RESEARCH AREA:	Nutrition, Physical Fitness & Rehabilitation
PRINCIPAL INVESTIGATOR:	Ronenn Roubenoff, M.D.
ORGANIZATION:	Tufts University
PROJECT TITLE:	Timed Feeding and Resistance Training to Prevent Muscle Atrophy

Project Executive Summary

Resistance training (RT) is one modality that offers the hope of mitigating or reversing muscle loss induced by weightlessness. Recent studies suggest that the timing of feedings around a bout of RT may maximize the effect of exercise on muscle protein balance. We therefore propose to test whether timed feedings, given before RT sessions, is superior to RT alone as a countermeasure against muscle atrophy induced by bed rest. This proposal is directly applicable to the NSBRI Nutrition, Exercise and Rehabilitation Team's first Focused Research Question, on Exercise Countermeasures. We hypothesize that:

1. The combination of timed feeding of an amino acid (AA) and sucrose mixture with RT (TFRT) is more effective than RT or feeding alone as a countermeasure against bed-rest induced muscle atrophy.
2. TFRT accelerates recovery from 28 days of bed rest compared to either RT alone or feeding alone over 14 days following strict bed rest.
3. TFRT works at least in part by modulating muscle levels of anabolic growth factors and cytokines IGF-1, TGF- β , IL-15; and catabolic cytokines IL-1 β , IL-6, TNF- α .

We will study healthy adult men aged 30-55, with BMI 23-28 kg/m², who will undergo 28 days of strict bed rest followed by 14 days of standardized weight-bearing physical activity on a metabolic ward. Subjects will eat 15 percent less than their energy requirements to replicate space-based anorexia. Subjects will be randomly assigned to one of three groups for the six-week study period: a) a sucrose/AA supplement given every other day (timed feedings given every 48 hours [TF group], which will be the control group for the study); b) RT every other day (RT group), with the supplement provided 24 hours after the RT to insure that the three groups are isoenergetic and isonitrogenous; c) RT every other day with the supplement given five minutes before the RT session (TFRT group). Outcomes will include body composition measured at multiple levels: total body potassium [TBK]; body fat and bone by dual-energy x-ray absorptiometry [DEXA]; bone turnover using urinary markers; regional muscle (mid-thigh muscle) computerized tomography [CT]; and cellular level (histochemistry of muscle fiber area and type distribution). We will also examine muscle strength and functional performance, and muscle gene expression of anabolic (IGF-1, TGF- β , IL-15) and catabolic (IL-1 β , TNF- α , IL-6, myostatin [GDF-8]) signals in muscles.

Earth-Based Application of Research Project

After prolonged bed rest and prolonged time spent in microgravity, significant deficits in muscle strength and muscle cross-sectional area may persist for weeks, suggesting that reambulation is not enough to recover function in a timely manner. The use of concentric and eccentric actions has resulted in strength preservation or enhancement, maintenance of muscle protein synthesis and prevention of fiber atrophy. An understanding of the recovery process can help design better rehabilitation strategies since recovery in the absence of rehabilitation is incomplete and muscle function is compromised up to weeks post-intervention. The development of an integrated nutrition and exercise countermeasure against the deleterious effects of immobilization will help.

RESEARCH AREA:	Nutrition, Physical Fitness & Rehabilitation
PRINCIPAL INVESTIGATOR:	Brian W. Tobin, Ph.D.
ORGANIZATION:	Mercer University School of Medicine
PROJECT TITLE:	Nutritional Modulation of Pancreatic Endocrine Function in Microgravity

Project Executive Summary

Our specific aims in this study are to: 1) assess the effect of a microgravity model cell culture on basal amino acid requirements and endocrine secretory function in human islets of Langerhans, and 2) determine human islet endocrine function while testing amino acid countermeasures in the microgravity model.

Ground-based and in-flight investigations illustrate changes in insulin, glucose, and amino acid metabolism in spaceflight. These observations may relate to altered pancreatic endocrine function which is insufficient to meet the needs of microgravity induced insulin resistance, and altered amino acid metabolism. The changes observed include decreased glucose tolerance, increased circulating insulin, and increased reliance upon glucose in muscles. The metabolic milieu resembles an insulin resistant syndrome, accompanied by a compensatory increase in pancreatic insulin secretion. However, the increase in insulin secretion is insufficient to ameliorate muscle atrophy. The increased insulin secretion is well correlated to muscle atrophy in spaceflight. The influence of these changes upon the loss of muscle mass and general endocrine metabolic state are not well established, however. Countermeasures which could modulate insulin and glucagon secretion in a compensatory manner to overcome insulin resistance and promote amino acid uptake by peripheral musculature might decrease muscle atrophy and reduce injury following re-adaptation to unit gravity.

We hypothesize that human pancreatic islets of Langerhans have an increased requirement for amino acids in microgravity. We further hypothesize, that supplementation with specific additional amino acids will augment, enhance and normalize insulin secretion, when spaceflight paradigm stressors known to decrease insulin secretion, are applied.

It is anticipated that these studies will further refine our understanding of human pancreatic amino acid requirements and endocrine regulation: phenomenon which may be limiting to extended-duration spaceflight missions. These studies will test countermeasures to augment pancreatic endocrine function, while considering both insulin and glucagon production in a way that will involve supplementation of diet with additional amino acids. These measures are ultimately aimed at improving spaceflight induced muscle atrophy, and ameliorating current re-adaptation constraints.

Key Findings

We have accomplished a part of Specific Aim 1: "To assess the effect of a microgravity model cell culture on basal amino acid requirements and endocrine secretory function in human islets of Langerhans."

Our results of experiments conducted this year in which human pancreatic islets of Langerhans were cultured in the HARV bioreactor and contrasted to controls show reveals the following key findings:

1. There is a tendency towards less glucose utilization in HARV-cultured islets of Langerhans
2. There is a tendency towards enhanced insulin secretion in islets maintained in the HARV,
3. We observed differential alterations in the pattern of amino acid utilization in the HARV,
4. Islet TNF production favors greater activity in the HARV cultures.

Impact of these findings on the hypotheses or requirements (technology), objectives and specific aims of the original proposal:

Observation A: The tendency towards decreased glucose utilization in HARV-cultured human islets of Langerhans, supports the hypothesis that microgravity is associated with a sub-clinical diabetogenic state. The observation of lesser glucose utilization in human islets is consistent with observations of rat islets cultured in the HARV system when contrasted to controls.

Observation B: The increased insulin secretion in the pancreatic islets cultured in the HARV suggests that islets are responding to some stimuli similar to that observed in insulin resistant states. It is well established that even in the face of severe insulin resistance, and decreased uptake of amino acids by muscle in diabetic individuals, the output of insulin by the pancreas is dramatically increased. This scenario is consistent with the observations in human pancreatic islets of Langerhans in the HARV microgravity model system.

Observation C. The differential pattern of amino acid utilization is consistent with the hypothesis that microgravity causes alterations in the pattern of metabolic substrate utilization. This is consistent with published data, and supports the hypothesis that the peripheral tissues are not the only sites of altered amino acid metabolism. The pancreatic islets of Langerhans also appear to be altered in their patterns of metabolite use when cultured in a microgravity model system.

Observation D: The greater TNF production in pancreatic islets of Langerhans supports the hypotheses that insulin secretion is suppressed from reaching an adequate level sufficient to overcome peripheral insulin resistance in muscle tissue. That TNF can suppress insulin action is well established. That TNF is secreted by pancreatic islets of Langerhans was previously reported by our laboratory. Given that TNF in HARV cultures is increased, this scenario suggests that even in the face of a need for increased insulin secretion to overcome insulin resistance in muscle, that TNF may be suppressing a maximal beneficial response in the islets of Langerhans

Proposed research plan for the coming year

In the coming year we plan to accomplish a comprehensive analysis of the effects of: 1) microgravity simulation, 2) LPS, 3) epinephrine, 4) cortisol, and 5) amino acid administration, upon endocrine and cytokine function as well as the nutritional utilization of glucose, amino acids, and fatty acids.

We will accomplish this by carrying out studies according to the following description.

1) Approximately 24,000 human cadaveric pancreatic islets of Langerhans are isolated and purified by collaborator JRT Lakey, PhD, at the University of Alberta and shipped in Medium-199 to Mercer University School of Medicine (MUSM).

2) At MUSM, human islets of Langerhans are prepared for bioreactor culturing by collaborator SK Leeper-Woodford, BW Tobin, PhD, and PN Uchakin, with the technical expertise of Cynthia

Bruin, and are allocated into 10 ml disposable High Aspect Ratio Vessels (HARV) or standard 10 ml cell culture plates.

3) The 24,000 human islets of Langerhans are divided into independent variable group.

EFFECTS OF STRESSORS

HARV w/ LPS	HARV w/ Cortisol	HARV w/ Epinephrine	HARV w/ no treatment
Plate w/ LPS	Plate w/ Cortisol	Plate w/ Epinephrine	Plate w/ no treatment

EFFECTS OF AMINO ACIDS

HARV with Arginine	Plate with Arginine
HARV with WOLFE Amino Acids	Plate with WOLF Amino Acids

4) The islets are cultured for 48 hours and samples are taken at 0, 3, 6, 12, 24 and 48 hours for analysis of metabolites by our collaborative group. e) Aliquots of islet medium are frozen at -70°C and are subsequently shipped to the following collaborators for dependent variable analysis.

Dr Uchakin (MUSM): glucose, lactate, insulin, glucagons

Dr Leeper-Woodford (MUSM): TNF-alpha, Il-1, Il-6, NF kappa beta

Dr. Walzem (TAMU): fatty acids, lipids,

Dr. Smith (NASA-JSC): amino acids, nitrogenous compounds

5) Data are returned from the collaborators to MUSM and are analyzed by Drs. Uchakin, Tobin and Leeper-Woodford. All members of the PI and Co-I and add-on project team confer on data analysis and interpretation. All project team members are eligible for inclusion as authors on any or all abstracts, presentations, or manuscripts resulting from these studies. NSBRI is properly acknowledged in all presentations and publications.

RESEARCH AREA:	Nutrition, Physical Fitness & Rehabilitation
PRINCIPAL INVESTIGATOR:	Robert R. Wolfe, Ph.D.
ORGANIZATION:	The University of Texas Medical Branch - Galveston
PROJECT TITLE:	Skeletal Muscle Response to Bed Rest and Cortisol Induced Stress
END DATE:	06/30/2004

Project Executive Summary

Specific Aims

Prolonged space flight causes a loss of skeletal muscle mass that is detrimental to physical function, and amelioration of this response is essential for successful prolonged missions. There are two components of the loss of muscle mass in space flight. Prolonged muscular inactivity causes a reduction in protein synthesis, while at the same time stress (mediated by moderate hypercortisolemia) accelerates the rate of muscle protein breakdown, at least insofar as it relates to the rate of synthesis. Our previous work in normal volunteers has shown that a supplement containing a mixture of essential amino acids and carbohydrate stimulates muscle protein synthesis. Further, whereas ingested amino acids normally do not affect the rate of muscle protein breakdown, they limit the accelerated rate of breakdown that occurs in stress states, such as in severely burned patients. Consequently, we anticipate a mixture of essential amino acids (15g) and carbohydrate (30g) given as a supplement three times per day will limit the loss of muscle, and in turn muscle function, during our model of space flight, which is prolonged bed rest + hypercortisolemia. We are testing the hypothesis that essential amino acid/carbohydrate supplementation will ameliorate the loss of lean body mass and muscle function that occur after 28 days of bed rest, while improving nitrogen balance over the duration of the experiment. Further, we have quantified muscle amino acid and protein kinetics at the beginning and the end of bed rest in order to gain insight into the mechanisms responsible for the loss of muscle mass in untreated subjects, as well as into the mechanisms by which supplementation serves to decrease muscle catabolism. Specifically, we have determined muscle amino acid and protein kinetics over 24 hour periods before and at the end of bed rest in order to address the following hypotheses:

1. The normal anabolic response of muscle to a meal diminishes with prolonged inactivity and stress.
2. An amino acid/carbohydrate supplement will stimulate net muscle protein synthesis over the one-hour immediately following ingestion.
3. The normal anabolic effect of meals will not be affected by prior ingestion of a supplement.
4. The post-absorptive nadir in net muscle protein synthesis will be no greater in subjects receiving supplementation than in control subjects.

Key Findings

We have completed the study of seven subjects on the essential amino acid/carbohydrate supplement (EAA group) and six subjects receiving placebo (Placebo group). The findings indicate that supplementation with EAA maintains lean body mass (LBM) throughout 28 days of bed rest, while the placebo group experiences a loss of LBM. The EAA supplement maintains LBM by stimulating net muscle protein synthesis to a much greater extent than meal ingestion alone. Although this stimulation is diminished with increased inactivity, the net gain in muscle protein is still significantly greater than that produced by meals alone. In other words, even though the anabolic response to the EAA supplement decreases after 28 days of bed rest, it is still capable of producing a significant increase in net muscle protein synthesis.

Though EAA supplementation is capable of maintaining LBM, it does not maintain muscle strength. Measures of leg muscle strength decline after 28 days of bed rest despite the preservation of leg lean mass. These findings demonstrate that the maintenance of LBM alone is insufficient in terms of muscle function. Apparently, some neuromuscular component is also required to preserve muscle strength and function.

Our findings also demonstrate that the EAA supplement is capable of stimulating net protein synthesis when given in a stressed state, as simulated by cortisol infusion. The presence of elevated blood cortisol induces a loss of muscle protein even when a meal is given. Though the EAA supplement can slow this loss, it only does so temporarily, such that within one-two hours after the supplement, the muscle protein balance is again catabolic. After 28 days of inactivity, the response to a meal during elevated cortisol is further diminished, such that the muscle is dramatically catabolic. The EAA supplement is not capable of eliciting an anabolic response in the muscle after 28 days of bed rest. On the contrary, when the supplement is given without the presence of cortisol, the net effect is muscle anabolism over the study time period.

Impact of Findings

Our findings demonstrate that the anabolic response to a meal diminishes with prolonged inactivity and a stress challenge. The stimulation of net muscle protein synthesis immediately following each EAA supplement translates to a maintenance of muscle protein over a 24 hour period, and in turn, over the 28 days of bed rest. However, the maintenance of LBM does not translate to maintenance of muscle strength. The interaction of inactivity and stress exacerbates the ineffectiveness of ordinary meals. Though the EAA supplement can offset muscle catabolism during the stress state, the response is transient and incapable of ameliorating the overall loss of muscle protein. Taken together, these findings indicate that a nutritional supplement alone can reduce the muscle atrophy associated with space flight. However, whereas muscle mass can be maintained with a specified nutritional intervention, other modalities are required to preserve muscle function.

Proposed Research Plan

We are currently studying the effects of chronically elevated cortisol throughout 28 days of bed rest on muscle protein, LBM, and muscle function. As of this writing, we have completed 1 subject and are currently studying two more. This investigation will determine the interaction of chronically elevated cortisol and muscular inactivity on the loss of LBM and muscle function. This investigation will help determine an optimal operational countermeasure that can be economically (in terms of crew time and payload) utilized to ameliorate muscle loss during prolonged space flight.

RESEARCH AREA:	Nutrition, Physical Fitness and Rehabilitation
PRINCIPAL INVESTIGATOR:	Robert R. Wolfe, Ph.D.
ORGANIZATION:	University of Texas Medical Branch
PROJECT TITLE:	Nutritional Countermeasures to Ameliorate Losses in Muscle Mass and Function

Project Executive Summary

This project will investigate interactions between nutritional and exercise countermeasures as they relate to the loss of inactivity-related muscle mass and function. We propose to expand upon our previous studies demonstrating that daily supplementation with an essential amino acid solution preserves net muscle protein synthesis and in turn, lean body mass. The preservation of lean body mass also ameliorated the loss in muscle function; however, muscle function was not entirely preserved. Thus, our data indicates that a neuromuscular component is also required to preserve both muscle mass and function.

We propose to study an enhanced essential amino acid supplementation and hypothesize that a formulation high in leucine will stimulate synthetic mechanisms in a more efficient manner to maintain muscle mass. A ground-based model designed to more closely mimic the hormonal and muscular activity alterations in space flight will be used.

NSBRI RESEARCH PROGRAM RADIATION EFFECTS

Team Leader:	Dicello, J. F.	Hopkins/SOM		
Associate Team Leader:	Vazquez, M. E.	Brookhaven		
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RESEARCH AREA:	Radiation Effects
PRINCIPAL INVESTIGATOR:	Polly Y. Chang, Ph.D.
ORGANIZATION:	SRI International
PROJECT TITLE:	Charged Particle Radiation-Induced Genetic Damage in Transgenic Mice

Project Executive Summary

Evaluations of risks involving alterations in the genome using whole animal systems are essential to missions in space. The *lacZ* transgenic mouse model is the only system available, to date, for the assessment of alterations in the genome in every tissue of the animal. In this model system, every cell of the animal contains multiple copies of an integrated but inert target transgene. Radiation-induced mutations can be measured and specific genetic alterations characterized using established protocols. Genetic alterations in tissues that are of high priority in NASA's Strategic Program Plans, but are not accessible using conventional techniques, e.g., the central nervous system, can be evaluated using this model system. In addition to measuring short (one week) and long term (up to 16 weeks after treatment) mutagenicity in the reporter transgene, concomitant evaluation of the clastogenic potential of highly charged and energetic (HZE) particle radiation can also be done using the same experimental animals. Some of these include examining radiation responses in the hematopoietic system by enumerating micronuclei (MN) in peripheral blood, evaluating chromosomal damage in either circulating or bone marrow lymphocytes by using fluorescence *in situ* hybridization (FISH) techniques, and induced gene expressions in tissues by using RT-PCR. Specifically, our research aims for this project include the use of *lacZ* transgenic mice to characterize the dose- and time-dependent radiation-induced responses in *lacZ* transgenic mice after high Linear Energy Transfer (LET) iron particle beams generated at Brookhaven National Laboratory and low LET proton irradiation at the Loma Linda University Medical Accelerator. We will measure the initial effects and long-term residual consequences of radiation exposure in tissues that are of high priority to NSBRI and NASA, namely the brain (CNS) and compare these responses to another tissue such as the spleen that is known to be a highly proliferative tissue with stem cell populations. We hypothesize that the *lacZ* mutation frequency (MF) in individual tissues will increase as a function of dose for each tissue, that this response is LET dependent, but the level of induction of MF is dependent on the specific tissues analyzed. Micronuclei (MN) in peripheral blood have been used extensively as a biomarker to evaluate radiation toxicity in the human population. We aim to examine radiation responses in the hematopoietic and lymphatic system in the same experimental animals. We expect that the level of genetic damage as well as the kinetics of removal of aberrant cells and the spectrum of chromosome aberrations is dose dependent.

Variations in genetic background have been shown to impact an individual's sensitivity to radiation exposure. The tumor suppressor *p53* gene function has been shown to be radiation responsive and very important in the regulation of cell growth, proliferation, differentiation, and apoptotic signaling pathways in many tissues. We cross-bred the C57 *lacZ* transgenic mice that are wild type for *p53* (*p53*+/+) with *p53* nullizygous (*p53*-/-) mice to establish breeding colonies of transgenic mice, all possessing the *lacZ* transgene and are either hemizygous or nullizygous for *p53*. These animals will be used to assess tissue-specific *p53*-dependent (or -independent) molecular and genetic mechanisms in radiation-induced damage resulting from exposure to particle beams in the energy range relevant to space radiation. Specifically, animals with different *p53* genetic backgrounds will be exposed to a range of doses of either iron particles or proton radiation and tissue-specific radiation responses using the same endpoints as mentioned in the previous section will be evaluated. Results from these studies will reveal the impact of

variation of genetic background to an individual's sensitivity to radiation exposure of different LETs.

Research activities for our second funded year include characterizing the dose and temporal dependence of iron-particle induced genetic damage in the *lacZ* transgenic mice and continued efforts to analyze samples archived from animals exposed to proton exposure.

We have previously reported that protons are effective in inducing dose-dependent increases in the levels of micronucleus in immature reticulocytes (MN-RET), a well accepted marker for genetic toxicity, in peripheral blood of mice. This effect appeared to saturate at higher doses. Animals exposed to an acute 4 Gy proton dose showed symptoms of transient systemic toxicity as evidenced by a dramatic drop in total reticulocyte counts shortly after radiation treatment. The percentage of MN-RET also varied as a function of time (24 - 120 hours) after exposure and in most animals, complete recovery was observed at one week after exposure. New evidence gathered during the Spring 2002 showed that the levels of MN-RET in peripheral blood in animals exposed to a range of 1 GeV/amu Iron ions were significantly higher than those from the controls at doses ≥ 0.5 Gy as early as 17 hours after exposure. The MN-RET levels returned to control levels for lower doses within three days, but were delayed up to one week post exposure for animals exposed to higher doses. Fluence-based analysis of the MN-RET data revealed that the relative effectiveness of iron ions in inducing peripheral blood MN-RET appears to be similar to those of protons at this time point, suggesting that the RBE for MN-RET may be approximately one for this end point.

We have completed the analysis of proton radiation induced changes in *lacZ* transgene in the brain and spleen tissues at one, eight and 16 weeks after exposure. New information generated from spleen tissues at 16 weeks post irradiation demonstrated that mutant frequencies (MF) in the transgene, although still higher than spontaneous MF in the control animals, was reduced when compared to the eight week data for doses ≥ 0.5 Gy, suggesting that either the radiation induced damage to the genome was repaired or that the heavily damaged cells were eliminated from the cell population. Mutation results from the 16 week 0.1 Gy irradiated spleen tissues were interesting in that the MF was persistently higher than their age-matched sham-treated controls. These results suggest that tissue recovery mechanisms after low dose exposures may be different from those that were exposed to high radiation doses and warrants further investigation.

LacZ MF in the brain tissues after proton exposure showed that proton exposures induced significantly higher transgene MF as early as one week after radiation exposure. Such elevated levels of MF persists for doses ≥ 0.5 Gy doses at the later eight and 16 weeks post irradiation when compared to the parallel sham-treated control animals. Although the overall MF in this tissue is lower than those obtained from the spleen at each of the time points, elevated MF at late time points in this tissue may be of particular concern to the Space Radiation Health community in terms of long term risk for the CNS tissue. These *in vivo* observations may provide important information regarding late tissue-specific effects that are not readily available in *in vitro* cell model systems.

Transgene MF in the brain and spleen tissues were measured after animals were exposed to an acute range of high LET Iron particle radiation. Preliminary results show that indeed, this *in vivo* model system is sensitive to the detection of HZE-induced genetic damage in tissues. In the brain, up to > 2 fold higher than spontaneous MF was observed in samples harvested from animals at eight weeks after they were exposed to a single high dose of 2 Gy. In the spleen tissue, this effect was less pronounced, with the increase in MF reaching a plateau at doses > 1 Gy. When compared with the proton results, the current findings suggest that induction of *lacZ* MF in tissues is not only tissue specific, temporally regulated but also dependent on LET of the incident radiation beam.

To address NASA's interests in examining potential countermeasures that will protect astronauts in long term space missions, we had initially proposed examining the effects of the pro-inflammatory cytokine IL-1, known to be a low LET radioprotectant, to alter the level of radiation-induced genetic damage after proton radiation. However, due to the reviewer's concerns regarding the use of this cytokine, we have redirected our research in this aim to study the use of currently available passive shieldings relevant to space travels. Animals were exposed to aluminum - or polyethylene-modified protons or iron irradiations and biological responses, including cytogenetic measurements as well as MF in the *lacZ* transgene were measured as a function of time and dose. Our hypothesis is that the profile of beam energy and charge will be modified as particle beams traverse through different materials. We have results showing that the MN-RET yield in peripheral blood, *lacZ* MF in the brain and spleen after 2 Gy of protons with or without aluminum shielding were the same, suggesting that aluminum did not afford any protection for the animals at this dose level, possibly due to effects of fragmentation of the proton beam in the metal. In the Spring 2002 BNL experiment, animals were exposed to a low (0.1Gy) or high (1 Gy) dose of 1 GeV/amu Iron particles with or without 10 or 15 cm polyethylene. Preliminary MN-RET results from these studies show no difference in the percentage MN-RET in peripheral blood between the shielded and their unshielded counterparts. These results are consistent with published observations made with shielding studies on human lymphocytes.

The construction of a new dedicated beam line at Brookhaven National Laboratory is now complete and we have obtained approved beam time for 1 GeV/amu Iron ions during the upcoming NSRL-1 run (October - November 2003). During this run, we aim to expose *lacZ* animals that are either *p53* hemizygous or nullizygous to a range of Iron doses (0.1 – 2 Gy) and monitor cytogenetic damage and recovery in these animals in both the circulating reticulocytes as well as the bone marrow compartments. Animals will also be housed up to 16 weeks after exposure so that late tissue responses can be measured using the transgenic mutagenesis assays. Our results obtained from these studies will be compared to the results we already have on hand for the *p53* wild type animals to determine the impact of *p53* genetic status on radiation sensitivity.

RESEARCH AREA:	Radiation Effects
PRINCIPAL INVESTIGATOR:	John F. Dicello, Ph.D.
ORGANIZATION:	Johns Hopkins University School of Medicine
PROJECT TITLE:	Radiation Effects Core Project: In Vivo and In Vitro Studies

Project Executive Summary

The risks of cancer to personnel in space from the naturally occurring radiations are generally considered to be one of the most serious biomedical limitations associated with long-term human space missions, as noted in two recent reports of the National Research Council/National Academy of Sciences. The paramount goals of the Radiation Effects Team for the National Space Biomedical Research Institute are: to determine carcinogenic consequences of radiations in space in an appropriate model; to develop effective physical and pharmaceutical countermeasures; and to study ways to reduce the risks of cancer and other diseases associated with such exposures.

During interplanetary missions, personnel in space will be exposed to galactic cosmic rays, including high-energy protons and energetic ions. (Ions with high energy, E, and atomic numbers, Z, greater than one are usually called HZE particles.) In addition, solar events will produce radiation fields of high intensity for short but irregular durations. The level of intensity of these radiations is considerably higher than that on Earth's surface, and the biological risks for carcinogenesis to astronauts are consequently elevated. Our group is examining the risk of cancers in model systems resulting from their low-dose exposures to photons, protons, and iron by using ground-based accelerators, which are capable of producing beams of such particles at energies comparable to those encountered in space. We have successfully conducted a series of experiments using a 1-GeV iron beam at the Brookhaven National Laboratory and 250-MeV protons at Loma Linda University Medical Center's proton synchrotron facility. As part of these studies, we have been collaborating with a companion project (David Huso, Principal Investigator) that is investigating the potential for anti-estrogen-based pharmaceuticals to reduce the risk of cancer after irradiation at the level of doses and for particle types expected in space. The hypothesis is that carcinogenesis in in-vivo models can be used to extrapolate to risk in humans and the risk of hormone-stimulated cancers such as breast cancer can be reduced by the subsequent administration of appropriate drugs after exposures to protons and densely ionizing radiations such as energetic heavy ions and neutrons. Additionally, the hypothesis is that the precursors of cancer can be altered at the promotion and progression stages of the diseases rather than the initiation. If this latter hypothesis is correct, it could reduce or eliminate the need for administering drugs in anticipation of significant exposures.

Theoretical studies carried out in a collaboration between scientists at NASA's Johnson Space Center and Johns Hopkins University are providing methods and predictions which are being used to assess the levels of radiation risks to be encountered, and to evaluate appropriate strategies for countermeasures. Continued collection and analysis of data from this project over the next three years will further enhance the precision of our estimates of biologic response and reduce the large uncertainties associated with previous assessments of risks for activities in space.

The research has consisted of four successive series beginning with a feasibility study to develop the logistics and infrastructure for these types of studies and followed by three studies to investigate the incidence of mammary carcinomas and the change in risk with the subsequent administration of Tamoxifen. The final three studies are still in progress, but the first one has

been successfully completed, and the data are being examined and analyzed. The initial results provide some of the first in-vivo data for the risk of cancer from both energetic protons and heavy ions. Initial results from the companion project indicate validation of the hypothesis that the risk can be reduced by subsequent pharmaceutical intervention with drugs.

Although the work in this project is primarily directed toward risks associated with space travel, the problem of protracted exposures to low-levels of radiation is one of national interest in our energy, defense, and homeland security programs, and the present results suggest new paradigms for addressing such risks.

RESEARCH AREA:	Radiation Effects
PRINCIPAL INVESTIGATOR:	David L. Huso, Ph.D.
ORGANIZATION:	Johns Hopkins University School of Medicine
PROJECT TITLE:	Chemoprevention and Radiation-Induced Neoplasms

Project Executive Summary

Chemoprevention is a pharmaceutical approach to arresting or reversing the process of carcinogenesis during cancer's typically prolonged latent period (often 20 years or more) before invasion or metastasis occurs. Surging scientific and public interest in applying chemoprevention strategies to people in the general population that have been identified to carry even slight increases in the risk of developing cancer (e.g. genetic risk) is fueling the identification of exciting new chemopreventive agents. Some now argue that future development of chemopreventive agents offers greater potential for the long-term control of cancer than the much more widely studied and aggressively pursued chemotherapy agents.

The major long-term risk associated with radiation exposure received during space travel is predicted to be radiation-induced cancer. The cancer-causing effects of low-LET radiations such as x-rays, g-rays, or electrons, typical of environmental earth exposures, have been relatively well-established. However, radiation likely to be encountered in space includes mainly heavy ions and protons along with their secondaries. Much less is known about the biology and risks associated with these types of radiation. The doses of radiation likely to be received even for long missions are probably low, but cover a broad range and are very unpredictable due to solar events. Like other types of radiation, the increased cancer risk associated with proton and heavy ion exposure is troubling because many radiation-induced cancers do not appear until later in life. Therefore, a large amount of uncertainty exists in how best to assess and manage the radiation risks associated with space travel.

Two high priorities in preparation for long term missions are 1) providing a better understanding of both the short-term and long-term carcinogenic risks of heavy ion or proton radiation and 2) developing pharmaceutical countermeasures to mitigate the carcinogenic risk associated with low-dose and mid-dose exposures to these types of radiation. Currently there are 3 cancer chemopreventive strategies that have clearly proven efficacy in preventing human familial and sporadic cancers 1) selective estrogen receptor modulators for prevention of breast cancer, 2) NSAID's (nonsteroidal antiinflammatory drugs) which may prevent a variety of cancers, and 3) retinoids for certain epithelial cancers. As countermeasures to the cancer risk associated with space travel, these chemopreventive approaches offer a particularly promising approach for countermeasure investigation because of: 1) these compounds are currently being used as preventives for human cancers although they are untested against proton or heavy ion-induced cancer, 2) there are difficulties associated with absolutely blocking radiation-induced mutagenic damage to DNA during prolonged space travel, either with shielding or pharmaceuticals, and 3) the prolonged latency period of most radiation-induced cancers (especially at low doses) offers a prolonged time period to administer chemopreventives. This is important since the latency period is the time when the most successful chemopreventives exert their effects. For most cancers, compounds that modulate the regulation of cell growth and apoptosis (rather than blocking mutagenic damage to DNA) have to date shown particular promise in preventing overt cancer from developing in susceptible organs.

Organs are not equally sensitive to the carcinogenic effects of radiation. Tissues that appear to be at higher risk for developing radiation-induced neoplasms include the female breast, the gastrointestinal tract (colorectal cancer), the thyroid, the bone marrow/lymphoid system

(leukemia), and the lung. Women have an increasing role in the space program. The female breast is particularly sensitive to the carcinogenic effects of radiation and therefore a relevant tissue in which to study chemoprevention of radiation-induced cancer. Chemoprevention of radiation-induced cancer in this sensitive target organ provides an excellent system in which to initially gain insights into the chemoprevention of radiation-induced cancer in general.

Over the past few years, tamoxifen has not only emerged as an effective chemopreventive against breast cancer, but it has also become the most widely prescribed anticancer drug in the world. It is a prototype of the group of pharmaceuticals called selective estrogen receptor modulators. Tamoxifen had been used for over 25 years for breast cancer treatment prior to its application as a chemopreventive. This level of acceptance for use in humans along with its proven chemopreventive efficacy against sporadic breast cancer provides a strong rationale for investigating its safety and efficacy against breast tumors induced by heavy ions and protons. As a potential countermeasure to the risks associated with prolonged space missions, the tamoxifen family of compounds have outstanding potential with a high level of readiness.

The class of compounds that includes tamoxifen, the selective estrogen receptor modulators (SERM's), are thought to have outstanding potential both in estrogen replacement therapy and as chemopreventive agents. Burgeoning research and development of new SERM compounds has led to many new and improved SERM's undergoing trials. Tamoxifen, however, remains the prototype SERM for breast cancer chemoprevention. Newer SERM's will hopefully further improve on tamoxifen's effects while reducing its side effects. SERM's are ligands for the estrogen receptor (ER) and modify carcinogenesis in breast epithelial cells by antagonizing ER signaling. However, in other tissues SERM's can act as partial ER agonists and promote the beneficial effects of estrogens in, for example, the skeletal and cardiovascular systems. Interestingly, tamoxifen may also affect carcinogenesis in a number of organ systems by disrupting apoptosis regulation in proliferating cells. In spite of the widespread use of tamoxifen, very little is known about its lifetime effectiveness against radiation-induced neoplasms-particularly those induced by radiation likely to be encountered in space such as protons and heavy ions.

In vivo studies provide a powerful means for directly evaluating the effectiveness of particularly promising chemopreventives against cancers that may occur following radiation exposure. The rat mammary tumor model has been used extensively to analyze the carcinogenic effects of both chemical xenobiotics and physical agents. The Sprague Dawley rat mammary tumor model is particularly well-suited for studies in the low dose range because this model is prone to develop induced mammary neoplasms early in life. Previous studies using the Sprague Dawley model have shown that sublethal doses of radiation (x-rays, gamma rays, neutrons-not particularly relevant to space travel) induce mammary tumors, often within one year, and with a linear dose-effect relationship. Thus the Sprague Dawley rat mammary carcinogenesis model not only closely resembles human breast cancer biologically, but it also is a highly sensitive model in which to examine the effects of radiation exposure and for testing pharmaceutical countermeasures against radiation effects. Our initial studies have focused on the effects of whole body, low level heavy ion and proton radiation along with chemoprevention of similarly induced mammary tumors using the female Sprague-Dawley rat mammary tumor model. The well-studied, widely prescribed, prototype SERM, tamoxifen has been effectively and safely used in humans for chemotherapy for almost two decades. These advantages, along with an understanding of its molecular mechanism of action, suggest it would be an excellent candidate for successful long-term chemoprevention of specific proton and heavy ion-induced cancers. The prospect for successful long-term chemoprevention of this potentially important, late-appearing cancer relevant to space radiation exposure is indeed an exciting prospect.

Hypothesis and Aims: There is an uncertain, but serious risk of cancer potentially associated with prolonged space travel. These risks cannot be addressed with shielding alone. Our first hypothesis is that modeling these risks can remove much of the uncertainty and would allow better management of some of the radiation-risks associated with prolonged space missions. Our second hypothesis is that the increased cancer risk that may be associated with radiation in the space environment can be mitigated by chemopreventive countermeasures implemented during the long cancer latency period that follows radiation exposure. The cancer causing effects of radiation as well as the safety and efficacy of chemopreventives have not been determined under conditions relevant to space. Animal models provide the best tools to test these hypotheses in relevant settings and should provide important insights into the chemoprevention of breast cancer in the general population.

Specific Aims:

1. To determine the relative risks associated with exposure to the types of radiation encountered in space using a sensitive *in vivo* model of radiation-induced cancer.
2. To determine if pharmaceutical cancer chemopreventives could provide a safe and effective countermeasure approach to mitigate the cancer risk that may be associated with exposure to the types of radiation likely to be encountered in space.

Key findings: Although our studies are not complete, *preliminary trends in our tamoxifen studies have pointed to a proof of principle for a strategy in which chemopreventive agents could play an important role in preventing breast cancer following exposure to radiation during space travel.* Confirmation of these trends is still pending the completion of these studies. Since Dr. Huso took over as PI of the chemoprevention studies, considerable progress has been made in this area. Since cancer chemoprevention in general is still in its infancy as an emerging field, chemoprevention based on new targets and emerging compounds, hold considerable promise for continued improvement of strategies to effectively mitigate risks associated with radiation and other predisposing factors for cancers. During the coming year we plan to complete our studies on tamoxifen chemoprevention of radiation-induced cancer and analyze in detail the overall findings. The data should give new insights into the use of pharmaceuticals in the mitigation of radiation-induced cancer.

The implications of our findings for future research and risk reduction for both space exploration as well as for the general population. The implications are clear. Our results, though preliminary, provide a glimpse of the enormous potential payoff that chemoprevention research could provide not only for the future health of astronauts exposed to radiation, but also in the general population in the battle against cancer. Regardless of the reason for an individual to be at increased risk for developing particular cancers, be it radiation exposure as in our studies (relevant to astronauts and space travel) or genetic and environmental factors (relevant to the general population), specific chemopreventive compounds and strategies can be identified and implemented to mitigate risks that predispose individuals to cancer. Much work remains to be done to fully realize the benefits of chemoprevention strategies specifically in the battle against radiation-induced cancer. Support for research into chemoprevention of radiation-induced neoplasms such as that provided by NSBRI therefore benefits not only space exploration efforts, but what is learned in this important area also could provide unique insight into cancer chemoprevention for the general population.

RESEARCH AREA:	Radiation Effects
PRINCIPAL INVESTIGATOR:	Ann R. Kennedy, Ph.D.
ORGANIZATION:	University of Pennsylvania School of Medicine
PROJECT TITLE:	Countermeasures for Space Radiation Biological Effects
END DATE:	05/31/2004

Project Executive Summary

It is our hypothesis that control of radiation-induced oxidative stress will reduce the risk of cancer development. In this grant work, there are two major specific aims: to determine whether certain dietary supplements can reduce space radiation induced oxidative stress in cultured human cells, and to determine whether the dietary supplements shown to reduce oxidative stress in cultured cells have effects on space radiation induced oxidative stress in animal model systems. The supplemental agents evaluated alone and in combinations include: vitamins C, E, folic acid, glutathione, N-acetyl cysteine (NAC), selenomethionine (SeM), alpha-lipoic acid, niacin, thiamin, Co-enzyme Q10 and the soybean-derived Bowman-Birk inhibitor.

The levels of oxidative stress in cultured cells are determined by a dichlorofluorescein fluorescence assay, which has been adapted for evaluation of the effects of space radiations as part of this project. In animals, the total antioxidant status (TAS) in serum or plasma samples is used as the indicator of oxidative stress. The agents shown to reduce space radiation-induced oxidative stress in vitro (in MCF10 cells, a human breast epithelial cell line) include: vitamin C, vitamin E succinate, NAC, SeM, alpha-lipoic acid (in the reduced form) and Co-enzyme Q10. The effects of treatment with this combination of dietary supplement agents on bio-reduction capacity in animals were evaluated in rats irradiated with iron ions, protons or gamma-rays at a dose of 200 cGy. Sham-irradiated animals were used as controls. The results demonstrated that the TAS decreased in the irradiated animals fed with the control diet.

The control animal diet (AIN-93G) contained many of the nutritional supplements at levels comparable on a weight basis to the human RDA levels. Supplementation of the diet with the nutritional supplements prevented the drop in the serum or plasma TAS after the radiation exposure. These results suggest that diet supplementation was effective in preventing the reduction in TAS in the animals irradiated with all three types of radiation. These results indicated that both serum and plasma samples are suitable for a determination of TAS in the irradiated animals.

The in vivo and in vitro results described above address the two major specific aims of this research project, and they indicate that the dietary supplement agents evaluated can reduce the levels of radiation induced oxidative stress both in vitro and in vivo.

The final year of this project represents a no-cost extension of the grant which ended on May 31, 2004. During the no-cost extension period, projects are being performed to optimize an in vivo assay for the measurement of radiation induced oxidative stress in animal tissues at highest risk for the development of radiation induced cancer (breast, thyroid, etc.). While the assay that has been used for the measurement of oxidative stress in vivo works well for plasma or serum samples, it has not yet been optimized for use in organs like the thyroid. By the end of this no-cost extension of our work, it is hoped that we will have an assay method that has been validated for the measurement of radiation-induced oxidative stress in many different organ systems.

Earth-Based Applications of Research Project

Radiation-induced oxidative stress is almost instantaneous with the radiation exposure and thus precedes most, if not all, of the other radiation induced short-term and long-term biological effects. Thus, countermeasures for radiation induced oxidative stress are likely to be very useful for preventing radiation-induced tissue damage, and presumably cancer development. The countermeasures for radiation-induced oxidative stress developed as part of this research program have significance for all individuals exposed to ionizing radiation on earth as well as in space. The countermeasures shown to protect against radiation induced biological effects from space radiation will be equally useful for protection against radiation induced biological effects on earth.

RESEARCH AREA:	Radiation Effects
PRINCIPAL INVESTIGATOR:	Marcelo E. Vazquez, M. D., Ph.D.
ORGANIZATION:	Brookhaven National Laboratory
PROJECT TITLE:	CNS Damage and Countermeasures

Project Executive Summary

Space travel beyond the Earth's protective magnetic field (for example, to Mars) will involve exposure of astronauts to irradiation by high-energy nuclei such as ^{56}Fe (HZE radiation), which are a component of galactic cosmic rays. These particles have high linear energy transfer (LET) and are expected to irreversibly damage cells they traverse. Exposure to HZE radiation may therefore cause progressive deterioration of brain function, adding to other inescapable damage involved in normal aging.

We are studying the hypothesis that long-term behavioral alterations are induced after exposure of the brain to 1 GeV/n iron particles with fluences of one to eight particles/cell targets. Previous studies support this notion but are not definitive, especially with regard to long-term effects. Our principal goal is to examine the neurological effects of high-LET radiation on C57BL/6 mice using a series of behavioral tests to unveil the temporal expression of altered behaviors in the radiation response, as well as the means, which can modulate these responses.

The studies are designed to:

1. Characterize the behavioral consequences after exposure to low-fluences of heavy ions and protons on C57BL/6 mice. The main behavioral endpoints to be used in these studies are locomotor activity to evaluate the integrity of striatal dopaminergic pathways, and spatial reference memory to probe hippocampal cholinergic pathways.
2. Characterize the neurochemical and structural changes induced by heavy ions and protons.
3. To develop countermeasures to protect neural cell populations exposed to low fluences of heavy ions and protons.

The project will test methods to protect injured neural cells based on their molecular and cellular mechanisms that may regulate neural cell survival in the central nervous system. Among the methods that will be studied is the direct administration of neuroprotective molecules as well as the modulation of apoptotic pathways by pharmacological manipulation. The effects of three different neuro/radioprotectors (GM1, melatonin and PTF- α) on the levels of radiation induced neurochemical and structural damage will be compared with the level of behavioral alterations to determine a cause/effect relationship.

RESEARCH AREA:	Radiation Effects
PRINCIPAL INVESTIGATOR:	Marcelo E. Vazquez, M. D., Ph.D.
ORGANIZATION:	Brookhaven National Laboratory
PROJECT TITLE:	Risk Assessment and Chemoprevention of HZE Induced CNS Damage

Project Executive Summary

Because successful operations in space depend on the performance capabilities of astronauts, radiation-induced neurological damage could jeopardize the successful completion of mission requirements as well as have long-term consequences on the health of astronauts. It is therefore necessary to understand the nature of this risk in order to assess its seriousness and to develop countermeasures.

Compared to the large literature associated with radiation therapy, knowledge is limited about the cellular and molecular responses of cells to high-LET HZE radiation in general and very limited about the central nervous system (CNS) specifically. Therefore, we will compare the effects of charged particle (Fe, Si), protons, gamma and x-ray radiation on the cells of the CNS, namely neurons and glial (astrocytes and oligodendrocytes). Cell cultures of CNS cells, both cycling and post-mitotic differentiated cells, will be utilized as model systems. We will test the hypothesis that exposure to low fluences/doses of heavy ions and protons can induce cell death in neural CNS neural cells and that increasingly dense ionizing radiation will be increasingly toxic.

The activation of two separate stress signal transduction pathways will be examined (p53 and ceramide) for their role in causing cell death or other deleterious changes caused by irradiation. And with respect to p53, we will determine which of the post-translational modifications in regulating p53 function are relevant for charged particle induced cell death. Finally, we will test the hypothesis that modulating the stress signal transduction pathways will modify the radiation response of brain cells exposed to heavy ions and protons, and test the efficacy several compounds as potential countermeasures for HZE radiation toxicity.

NSBRI RESEARCH PROGRAM SMART MEDICAL SYSTEMS

Team Leader:	Crum, L. A.	Washington		
Associate Team Leader:	Soller, B. R.	UMass		
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RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Lawrence A. Crum, Ph.D.
ORGANIZATION:	University of Washington
PROJECT TITLE:	Guided High Intensity Focused Ultrasound (HIFU) for Mission-Critical Care
END DATE:	07/31/2004

Project Executive Summary

Specific Aims

The principal objective of this NSBRI Smart Medical Systems Team project is to develop an image-guided ultrasound therapy system for mission critical care. In long-term space flight missions, a number of medical situations could develop that if not adequately addressed would result in mission failure. For example, although gravity is significantly reduced in space, inertia is not, and the collision of an astronaut with a heavy object could result in blunt internal trauma and is often associated internal bleeding. In addition, as recent experiences in Antarctica demonstrate, medical conditions that require some form of surgery may well appear without warning, even when extensive pre-screening is undertaken. We are developing a smart medical device that will provide a versatile capability to treat a variety of these mission-critical medical conditions. We have demonstrated that a device that produces High Intensity Focused Ultrasound (HIFU) can be combined with a device that provides ultrasound imaging to produce a duplex system that can both image a particular condition of interest and provide therapy to relieve the condition. "Image-Guided Therapy" provides enormous potential for the treatment of a variety of medical conditions. In addition, we have demonstrated that the components of such a smart medical system can reasonably be expected to be lightweight and portable.

The Specific Aims of this effort are as follows:

Specific Aim 1: To develop a combined ultrasound guidance and therapy system.

This first-generation system should have the following components: (A) Laptop computer control, (B) Software control, (C) Compatibility with commercial ultrasound imaging systems, (D) Single element resonant transducer, and (E) Dynamic depth focusing.

Specific Aim 2: To perform studies on the combined system that would lead to optimal performance parameters.

Among the studies to be performed are the following: (A) Biological effects, tissue necrosis, acoustic hemostasis, (B) Image quality for diagnosis, (C) Targeting and monitoring capabilities, (D) Acoustic focusing and power requirements, and (E) Thermal focusing limitations.

Specific Aim 3: Utilizing the results of Aims 1 & 2, to develop an integrated ultrasound guidance and therapy system.

This second-generation system would have the following characteristics: (A) Integrated imaging and HIFU therapy transducers, (B) Cavitation feedback, (C) Attenuation/thermal feedback to localize treatment site, (D) Perfusion and back-scatter imaging for treatment localization, and (E) Software control and friendly user interface.

Key Findings of the Project:

Our three Specific Aims have been met. Aim 1 was completed and reported in Year One. The ability to use ultrasound to guide HIFU is the foundation of the integrated system developed in Aim 3.

Aim 2 is the science to make the system clinically useful. Our group continues to lead the world in the field of acoustic hemostasis. The NSBRI project has leveraged other funding to use HIFU to stop bleeding. And we have found we can break kidney stones with HIFU. In particular the NSBRI research has led to the two most powerful ways to enhance or optimize tissue heating – short, strong pulses to exploit nonlinear wave propagation (initiated in Year 2) and dual-frequency mixing to exploit cavitation bubbles (discovered in Year 3).

Aim 3 has lead to the most portable and versatile guided HIFU system we know of. We have developed (A) Integrated imaging and HIFU therapy transducers, (B) Cavitation feedback, (C) Attenuation/thermal feedback to localize treatment site, (D) Perfusion and back-scatter imaging for treatment localization, and (E) Software control and friendly user interface.

Key Findings of Year 3:

We had three key findings in Year 3.

First, we found we could integrate the three key findings of Year 2 into a useful part of our system and expand of those findings. The findings were not solely academic discoveries but became enabling technologies. The protocol of using short high-amplitude pulses has been integrated in the system. We showed that doubling the acoustic amplitude of the source cuts the treatment time by more than one half. In Year 3, we utilized this technology and defined the mechanisms underlying the effect. Two, our lesion identification algorithm has been improved to even measure temperature of HIFU heated tissue before a lesion even forms. This enables safe and accurate targeting. Tests are underway of the system connected to the HDI-1000 imager with this imaging capability. Three, the patenting and licensing process continues for our circuit to permit real-time synchronization of HIFU therapy with an arbitrary ultrasound imager. Commercial partners may soon be using the technology to develop even smaller and more powerful systems than we have developed.

Second, we developed a complete integrated guided system within a suitcase. The system could be synchronized with an arbitrary ultrasound imager, such as the Philips HDI-5000 on the ISS. Or we added in Year 3 to use a simplified Doppler imaging system to locate the HIFU focus on a major bleed by visual or audio guidance. This is an uncomplicated user-friendly device to rapidly and accurately treat a mission critical bleed.

Third, we discovered that by using two frequencies instead of one HIFU frequency, we could accelerate hemostasis times 25 percent. We completed a careful study numerically and experimentally in vivo and in vitro that showed how two frequencies can increase cavitation, enhance heat deposition and accelerate hemostasis.

Impact

The components now weigh less than 7 kg, down from more than 30 kg in Year 1, and 40 kg at the start of the project. They are packaged in a single chassis (a suitcase) and operate with the Philips HDI-5000 ultrasound imager on the ISS or whatever imager is chosen for long-duration missions (i.e., a Terason or a Sonosite imager such as used in the SMS project lead by Dr. J. Thomas). Doppler makes a simple rapid method stop major bleeds without even the use of the ultrasound imager.

We have established a protocol to sweep short-duration, high-amplitude pulses produced by the dynamic focusing transducer to treat large areas rapidly without complications from cavitation. This includes sealing exposed lung from blood and air leakage. These findings expand our trauma care capabilities, improve therapy efficiency, and minimize the power draw required by the device. When two frequencies are mixed, the enhancement is even greater. Bleeds stop 25 percent quicker. In addition, since heating and greater, higher volume bleeds can likely be

stopped. Lastly, since the heated region is broader, likely larger vessels can be treated than were previously possible.

We can use the same dual frequency system to treat kidney stones. Renal Stone Formation is Risk 12 on the Critical Path Roadmap, and the device we are currently building may be a method to comminute renal calculi that do form in space. Stone comminution is part of our renewed grant.

Thus, a practical flexible HIFU system for space use is nearing reality, and simultaneously, The University of Washington's Center for Industrial and Medical Ultrasound (CIMU), with NSBRI funding, is one of the leaders making high intensity focused ultrasound an Earth-bound clinical reality. An MRI-guided HIFU device received FDA approval for the treatment of uterine fibroids this year. The company employs a former student who graduated from our group and has made use of CIMU's published work. Devices in China have treated more than 10,000 patients for cancer and are now negotiating exports to selected sites. CIMU has been consulted on these negotiations. Smaller hemostasis devices are being developed by two spin-off companies from CIMU. These new companies are beginning to generate the substantial private funding necessary to overcome the production, regulatory and insurance reimbursement challenges of a new therapeutic modality.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Lawrence A. Crum, Ph.D.
ORGANIZATION:	University of Washington
PROJECT TITLE:	Smart Therapeutic Ultrasound Device for Mission-Critical Medical Care

Project Executive Summary

Recent funding restrictions, events, and developments have forced NASA to eliminate the capability of a medically equipped rapid transit patient vehicle; accordingly, in medical emergencies, there has been a shift in ISS medical operations from a “stabilize and transport” approach to the current “stand and fight” mode of operations. In addition, plans for extended missions outside of Low Earth Orbit (LEO) must also follow this new “stand and fight” paradigm. With interest in the safety of astronauts, a number of risks associated with the Bioastronautics Critical Path Roadmap (CPR) become especially crucial. This project addresses four specific risks as described in the CPR as well as in the NSBRI Strategic Plan; viz., Risk No. 43 – Trauma and Acute Medical Problems; Risk No. 23 – Carcinogenesis Caused by Immune System Changes; Risk No. 38 – Carcinogenesis Caused by Radiation; and Risk No. 12 – Renal Stone Formation.

The principal, long-term objective of this effort is to develop a Smart Medical Device that would be lightweight, portable, FDA-approved, (some components or derivatives) under commercial development and capable of addressing the four risks described above. In particular, the device would be capable of utilizing diagnostic ultrasound imaging technology to detect a site of internal bleeding and the technology of High-Intensity Focused Ultrasound (HIFU) to rapidly elevate the local temperature to the point of cauterization, thus inducing (transcutaneous) hemostasis. In addition, utilizing a similar mode of operation, this device would be able to target a benign or malignant tumor and apply HIFU to the tumor to induce coagulative necrosis, thus performing bloodless ablation of the tumor without fear of metastasis. Furthermore, because the formation of renal calculi is a likely result in extended space flight, a relatively straightforward modification of the proposed device would be capable of detecting and comminuting a kidney stone sufficiently for it to pass without obstruction through the urinary system.

The principal objective of this effort is to develop a unique medical system that would generate new technology, as well as to exploit existing technology, to meet the specific and unique demands of human, long-term space flight.

Specific Aim 1: To expand the capability of our existing smart medical device for transcutaneous acoustic hemostasis to include a fully integrated ultrasound detection, targeting, and therapy system (CRL 5-6).

Specific Aim 2: To develop the capability of the integrated image-guided therapy system in Specific Aim 1 to target and ablate specific tissue volumes such as benign and malignant tumors (CRL 4-5).

Specific Aim 3: To develop the capability of the integrated image-guided therapy system in Specific Aim 1 to target and comminute renal calculi (CRL 3-4).

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Peter F. Davies, Ph.D.
ORGANIZATION:	University of Pennsylvania
PROJECT TITLE:	Vascular Genomics in Gravitational Transitions

Project Executive Summary

Aims

When changes in the biomechanical environment of the circulation occur, blood vessels undergo well-orchestrated structural and metabolic remodeling to restore optimal function. We propose that this remarkable adaptive ability lies at the center of orthostatic intolerance exhibited by most astronauts on return to earth's gravitational field after modest-to-long periods in microgravity. We are therefore mapping gene expression (transcription profiling) of the different vascular steady states exhibited *in vivo* (mouse) in simulated hypergravity and microgravity, and the transitions between them, in order to design better countermeasures for undesired vascular consequences in long-term space flight. In particular, the transition to hypergravity will simulate the effects experienced by astronauts upon return to a significant gravitational field (Earth, Mars) following adaptation to long periods of microgravity. The studies will generate a reference genomics database identifying gene expression changes in the arteries, heart and lungs induced by gravitational shifts and mining of such databases will provide a guide to potential countermeasures to offset deleterious effects.

Key Findings

During the first year we refined the antisense RNA techniques necessary to amplify RNA from small numbers of cells with high fidelity. This became necessary when it was apparent that no literature existed for a rigorous test of the protocols required in the mouse experiments. In a model experiment, vascular cells were stimulated with the cytokine TNF for which a small number of genes are known (through conventional Northern analyses) to change. RNA from the same pool was analyzed by microarray with and without amplification. Sophisticated bioinformatics analysis of 13,800 genes was performed. The data from unamplified and amplified RNA were analyzed for fidelity, sensitivity and utility. The expected prominent changes in known genes were detected in both groups with high retention of accuracy, an essential requirement for the proposed *in vivo* gravity experiments. An interesting additional and unexpected finding is that RNA amplification increased the detection rate of genes whose differential expression was just below a significance threshold in the unamplified assay i.e. greater sensitivity of detection of differential gene expression conferred by the linear amplification techniques employed. Most important, these differences were confirmed by real-time quantitative PCR of unamplified RNA. This work was published in the journal *Physiological Genomics* in April 2003. The studies were a prerequisite for the gravitational experiments because no such analysis existed that rigorously evaluated the accuracy of the transcription profiles arising from amplification of small amounts of blood vessel.

In extending the RNA amplification techniques we next addressed differential vascular cell gene expression in two sites in the aorta of the normal adult pig. Endothelial cell mRNA was isolated from two regions of the aortic arch characteristic of disturbed flow (pro-atherogenic) and undisturbed flow respectively. RNA from paired sites in individual aortas (n=8) was isolated, linearly amplified, reverse transcribed, and cDNA was hybridized to microarrays custom-prepared from the University of Toronto human cDNA cardiovascular database (~8000 genes) plus several thousand proprietary Incyte clones. Bioinformatics analyses identified expression patterns in the disturbed flow region indicative of an antioxidant endothelial profile that may be protective of a pro-inflammatory state. Some genes associated with major mechanisms believed

to initiate atherogenesis, eg pro-inflammation, were elevated but the critical adhesion molecules necessary to initiate inflammation were not differentially expressed in this region, consistent with the absence of any pathology by histological assessment. *This is an intriguing result that demonstrates the power of this approach in identifying the interactions of multiple genes need to be considered in defining atheroprotective or susceptible situations.* As far as we are aware, these are the first high throughput array analyses of arterial endothelial gene expression directly obtained from discrete regions of blood vessel. When compared with several studies that have profiled the effects of different flow treatments on cultured (as opposed to *in vivo*) cells, many differences of gene pathways were noted. This work has been submitted to the journal *Proceedings National Academy of Sciences USA*. While these studies were performed with larger blood vessels (porcine) in order to obtain enough lining cells (endothelium), the cell numbers used are comparable to, in fact less than, those we will obtain from whole mouse aorta for the gravity studies. Techniques for the dissection of mouse blood vessels, RNA isolation and amplification has been verified under normal gravitational conditions. These evaluative experiments demonstrate that we can successfully perform the entire sets of protocols from tissue isolation to bioinformatics and gene annotation prior to the gravitational shift experiments at NASA-Ames Research Center.

Hypergravity and transitional procedures on 96 mice at NASA Ames, using the 24-foot centrifuge in close collaboration with Ames staff members, is under way. Arterial, heart, and lung tissues harvested will then be analyzed at Penn. The molecular biology is demanding and lengthy, and the bioinformatics is complex. As in the case of our publications to date, we are taking steps to ensure that the data are openly available to the widest scientific community.

Impact

New techniques addressing vascular genomics have been developed, tested, and have withstood critical peer review in leading journals in the field of genomics and biology. We are now implementing them in carefully designed experiments in which gravitational shift is the variable.

Plans for the Coming Year

For studies of hypergravitational changes, the facilities of the Chronic Hypergravity Exposure Centrifuge at NASA Ames are suitable for long-term exposure of mice at 3G to simulate return to earth (or landing on Mars surface) after long term space travel. The current experiments will provide the conditions for database development.

Mice are exposed to micro or hyper gravity for up to 28 days and the effects upon gene expression in the major arterial system, heart and lungs will be measured by the techniques outlined above. Reversal of the adapted condition will also be evaluated on a temporal basis.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Scott A. Dulchavsky, M.D., Ph.D.
ORGANIZATION:	Henry Ford Health System
PROJECT TITLE:	Minimally Invasive Diagnosis and Therapy of Microgravity Medical Contingencies

Project Executive Summary

The diagnosis and management of acute health problems in space is problematic due to limited training of the Crew Medical Officer (CMO), human and environmental factors, and a lack of reference of the changes in anatomy, disease presentation and therapy in micro-gravity. There is no planned radiological capability aboard the ISS, further complicating medical diagnosis in space. Recent terrestrial investigations suggest expanded clinical applications of ultrasound and laparoscopy with miniature instrumentation which have applicability for aerospace medicine.

Our research will initially determine the diagnostic utility of ultrasound and/or micro-laparoscopy in select health contingencies with high potential mission impact. These diagnostic modalities will then be used to facilitate minimally invasive, definitive surgical therapy of selected contingencies in animal models in ground-based and simulated micro-gravity scenarios. Optimal just-in-time training regimens and computer-based refresher modules for non-physician CMO's to accomplish these tasks will be developed. The unique constraints imposed by training and equipment limitations and the space environment require the development of novel diagnostic and therapeutic strategies for crew member health problems including the expansion of ultrasound and mini-laparoscopy.

Thoracic ultrasound, initially investigated by NASA as an alternative diagnostic modality for pneumothorax, has proven accuracy in terrestrial and micro-gravity applications and will have wide spread impact in acute care on Earth in the future. Although some of the techniques investigated in our research are appropriate only for a micro-gravity environment, the majority of the diagnostic and therapeutic algorithms are readily transferable to terrestrial medicine including rural and military applications. The expanded use of the diagnostic and training modalities, if verified, would provide a significant, clinically relevant advance in space medical capabilities with profound Earth-based ramifications.

Main Findings in Reporting Year:

We have developed a multi-media, interactive program (OPE: Onboard proficiency enhancement) which allows just-in-time training paradigms to be used to rapidly train non-physician personnel in complex medical tasks. This training program has been used in the following experimental programs:

- Microgravity investigations on the KC-135: Non-medical personnel were able to successfully complete ultrasound and micro-laparoscopic investigations on animal models of space relevant disease.
- Ground-based studies: We have preliminary data which demonstrates that ultrasound can be performed by non-radiologists to diagnose dental and sinus pathology and musculoskeletal investigations.
- Space: The OPE program has been used by the ISS crews of Increment 8-11 to learn ultrasound.

Unique Claims of Study:

The modular development of an Onboard Proficiency Enhancement (OPE) program has allowed complex science to be conducted on the ISS with minimal crew training requirements.

Earth-Based Applications of Research Project

The just in time training algorithms developed in this proposal can be used to rapidly train non-medical personnel to perform complex medical diagnosis and treatment. This could be used in rural, military, or third world countries to deliver high-quality medical care.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Mark S. Klempner, M.D.
ORGANIZATION:	Boston University
PROJECT TITLE:	Smart Medical System for Detection of Microorganisms

Project Executive Summary

The goal of this program is to develop a revolutionary, non-culture-based microbial detection, identification and quantification system that can be used as part of a Smart Medical System for exploratory space travel. Rapid detection and identification of microorganisms are critical to many military and civilian applications ranging from food and water safety monitoring, biological warfare agent detection and to diagnostic microbiology of human and other biological specimens. For long-term exploratory space travel there will be a critical need for a smart medical system to monitor the air and water supply for microbial contaminants, as well as an intermittent need for assessment of biological specimens from symptomatic astronauts.

Current microbial identification systems are based on the gold standard of *in vitro* culture or DNA/RNA fingerprinting. Both require considerable sample manipulation, delay in readout, are semiquantitative and subject to interfering substances and contamination, and require additional processing to resolve complex mixtures of microorganisms. This proposal involves the development of a novel smart medical system to detect and identify bacteria through the use of microsensors and includes three steps: 1) Development of "fingerprinting" phage display libraries which can detect, identify, quantify and discriminate bacterial species in environmental and biological specimens; 2) Application of phage displayed peptides and antibody fragments in a microarray to the surface of a microsensor to demonstrate the microarray microbial fingerprint response to selected bacterial species using optical readout and electronic MEMS resonator arrays and to characterize the sensitivity and specificity for detecting and discriminating between bacterial species using surface "fingerprints;" and 3) Development of algorithms from the microarray response for the real time identification and discrimination of bacterial species.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Babs R. Soller, Ph.D.
ORGANIZATION:	University of Massachusetts Medical School
PROJECT TITLE:	Noninvasive Measurement of Blood and Tissue Chemistry
END DATE:	03/31/2004

Project Executive Summary

The long-term goal of this project is to create a noninvasive and lightweight system for measuring blood and tissue chemistry through the skin. The technology is based on Near Infrared Spectroscopy (NIRS) and is targeted to work on subjects of any ethnic origin. The system developed measures three metabolic parameters: muscle pH, muscle PO₂ (oxygen tension) and blood hematocrit. The system can be part of smart medical systems used to identify and treat traumatic injury and assess fitness levels on Earth and in space. It can also assist in the development of more time-efficient exercise countermeasures.

We developed novel algorithms to simultaneously and non-invasively measure muscle pH, PO₂ and blood hematocrit. We demonstrated human feasibility of this measurement on 18 patients undergoing cardiac surgery. The measurement algorithms applied to individual patients were accurate enough to detect small changes in local peripheral perfusion resulting from reduced blood pressure and temperature-induced changes in metabolic demand, despite significant variation in body temperature. Physiological changes during cardiac surgery model mild shock. We expect seriously injured patients to have significantly larger variation in the non-invasively measured parameters.

We have also developed novel techniques to correct transdermally collected spectra for the interfering effect of skin color. The technique was shown to improve the accuracy of hemoglobin measurement on tissue-mimicking phantoms. We also have shown that we can derive the skin color correction factor from human subject spectra.

We found that the spectroscopic equipment and probe placement techniques used for the cardiac surgery study were not stable and precise enough to allow calibration equations to be developed across multiple subjects. With funding from the US Army Medical Research Command, we have designed and built new hardware to overcome these difficulties. We collected a new set of calibration data from cardiac surgery patients with this equipment and were able to develop multi-subject calibration models with accuracy near our target values. However, the range of pH and PO₂ values for these calibration models was limited compared to our original set of patient data, and too small for use in an exercise study. We have developed a new human-subject protocol using hand dynamometry and will use data from this study to develop calibration equations, which have the range required not only for exercise studies but for serious traumatic injury as well.

We developed two-layer phantoms to spectroscopically study fat layers over muscle. We also developed computer-based Monte Carlo simulation models to study how variability in optical coefficients for muscle and fat would impact human spectra. Input from the models helped in the design of a modified fiber optic sensor to measure muscle spectra beneath a thick fat layer. We also modified our optical hardware to achieve good spectral signal from muscle beneath

thick fat layers. The sensor and monitor have been successfully tested on phantoms with thick fat layers.

Monte Carlo simulation showed that for most subjects, no fat corrections would be needed for NIRS measurements made on the forearm. We also used Monte Carlo simulations and phantom measurements to evaluate potential fat correction algorithms. Work in the next year will focus on implementation of the most promising approaches for fat correction, culminating in a demonstration on human subjects.

NIRS-based noninvasive measurement of muscle pH, PO₂ and blood hematocrit has been demonstrated on human subjects and techniques are being implemented to ensure that these measurements are accurate for all subjects, irrespective of fat thickness or skin color. We expect application in the treatment of critically ill subjects and for assessing exercise intensity and muscle fatigue. Next year we anticipate beginning validation of the sensor using specific NASA exercise protocols. We will also begin a study of Emergency Room patients in shock to derive values for muscle pH and PO₂ which can be used to guide resuscitation through a smart medical system.

Earth-Based Applications of Research Project

This work will have direct Earth-based application. The prototype monitors we are developing will have application in emergency response vehicles, emergency rooms and hospitals. Pre-hospital applications include assessing the severity of shock and triaging multiple casualties, as well as providing a sensor for a smart medical system to guide resuscitation from hemorrhage. In the ICU, we expect that this monitor will find application in distinguishing between hemorrhagic and septic shock. The direct muscle application of interest to NASA for assessing fitness in space may be useful to assess success of physical therapy in rehabilitating patients with muscle injury or atrophy.

There is also general medical application for the diagnosis of anemia and if small and inexpensive enough, screening world-wide for malnutrition. There is also a possible application for the diagnosis of diabetic foot ulcers. A smaller version of this monitor could find use in the training of elite and weekend athletes.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Babs R. Soller, Ph.D.
ORGANIZATION:	University of Massachusetts Medical School
PROJECT TITLE:	Noninvasive Measurement of Blood and Tissue Chemistry

Project Executive Summary

Trauma and acute medical problems along with loss of skeletal muscle mass, strength and endurance, are some of the most serious risks facing astronauts during long-duration space flight. The measurement of two metabolic parameters, muscle pH and oxygen partial pressure (PO₂) can be applied in both areas. This project examines the hypothesis that near infrared spectroscopy (NIRS) is a platform technology that can provide noninvasive physiologic monitoring in support of multiple NASA needs. The immediate goal is to produce and test a small, lightweight medical monitor which utilizes NIRS to measure important metabolic parameters. Requisite precision and accuracy will be demonstrated for both male and female subjects of any ethnic origin. The device will perform equally well on the palm or the thigh, despite gender and weight-related differences in fat thickness. This novel technology is expected to be extendable to measurement on other parts of the body and for additional medical parameters.

One application will be the assessment of fitness before, during and after space flight. Preliminary studies will demonstrate accurate measurement of muscle pH and PO₂ on subjects performing variable work cycle tests. Monitors will be manufactured by a local company and provided to NASA Johnson Space Center for more extensive testing. Additional physiologic data will also be collected for use by NSBRI's Nutrition, Physical Fitness and Rehabilitation Team to continue the investigation of noninvasive muscle pH measurement for the development of time-efficient exercise countermeasures. Another application will be in a Smart Medical System for the assessment and treatment of trauma or injury which results in reduced blood pressure, blood flow or cardiac output. Values for muscle pH and PO₂ that can be used to assess severity of injury and guide treatment will be determined from a study of patients who enter the emergency room in shock. The NIRS noninvasive metabolic monitor is expected to have many applications for NASA. The system will have additional use on Earth for military and civilian personnel treating mass casualties. It can also be used in the hospital, ambulances and helicopters. As part of a Smart Medical System, advanced medical assessment and monitoring may become available to physicians in remote and rural areas, who may not have access to specialist expertise. The monitor is expandable to measure new chemistries by altering the calibration equations stored in computer memory.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	Jeffrey P. Sutton, M.D. Ph.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Near-Infrared Brain Imaging for Space Medicine
END DATE:	06/30/2004

Project Executive Summary

This project is part of the NSBRI Smart Medical Systems Team. It develops and applies a new technology, diffuse optical tomography (DOT; also called near-infrared spectroscopy and imaging, NIRS/NIRI), for portable non-invasive functional monitoring suitable for neuroimaging in space. The technology has potential capabilities to:

- Quantitatively assess physiological adaptation (e.g., changes in intracranial pressure and blood flow) associated with microgravity;
- Detect regional brain activity correlated with performance under altered circadian and mental loads;
- Provide remote clinical assessment, and;
- Guide treatment.

In addition to applications for space, the developments of this project have relevance to Earth medical research and care. For example, DOT is now being tested for monitoring stroke progression in patients with acute cerebrovascular accidents. The project brings together scientists, engineers and physicians at the Massachusetts General Hospital/Harvard-MIT Division Health Sciences and Technology, and medical operations personnel at NASA Johnson Space Center (JSC), to work collaboratively on the development and testing of DOT as a space relevant technology.

The original aims are to:

1. Refine current DOT technology to build an instrument with improved spatial and temporal resolution to detect brain activity non-invasively, and in real time, through the intact skull;
2. Validate the improved instrument using functional magnetic resonance imaging (fMRI), which is a standard technology, and test DOT as a portable brain imaging device for assessing motor and cognitive activity under normal and sleep deprived conditions in normal human subjects;
3. Assess DOT, along with optical coherence tomography (OCT), to non-invasively measure altered intracranial pressure (ICP) in neurological patients, given that altered ICP may occur in the space environment, and;
4. Refine a system for automated image interpretation using individualized brain models and computational techniques.

During the third and final year of this award, including supplementary extension, key findings and accomplishments include:

- Continued refinement of DOT instrumentation to validate the technology using fMRI;
- Simultaneous application of DOT and fMRI to detect brain activity differences during SpaceDock task performance (a custom-designed space-relevant visuomotor performance task);

- Simultaneous application of DOT and fMRI to detect brain activity differences associated with sleep deprivation;
- Manuscript completed for the sleep-deprivation application data (2 manuscripts under review);
- Further development of a demonstration system for automated multi-sensor analysis and interpretation (Automated Intelligent Medical System; AIMS), which can use sensor information from multiple astronauts and/or the environment to make medical diagnoses;
- Manuscript completed describing the AIMS computational system and characterization results, and;
- Leveraging of the NSBRI funding to win an R21 award to investigate in greater detail the application of DOT in patients with altered intracranial pressure.

The impact of these findings are that they address the technology requirements set forth in Aim 1, and provide validation for DOT as laid out in the objectives and hypotheses of Aim 2. The previously acquired motor data using simultaneous DOT and fMRI confirm one of the hypotheses contained in Aim 2, namely that DOT and fMRI will be able to detect changes in regional brain activity contralateral to motor movement. The DOT/fMRI findings using the SpaceDock task confirm the hypothesis that the DOT technique can detect frontal cortex differences as a function of mental load in normal and sleep-deprived subjects. The AIMS system provides a general-purpose platform for simultaneously and autonomously monitoring many sensors, and speaks to the issue of individualized, digitized human, anatomical brain models upon which time-derivative functional data are used for interpretation and display in real time (Aim 4).

In the coming year, under the new R21 funding, patient populations with elevated ICP will be investigated in considerable detail. Finally, continued development will take place on the AIMS informatics system towards more automated machine/human interface, applicable to DOT imaging as well as other, analog settings.

Earth –Based Applications of Research Project

Drs. Boas and Koroshetz have an active research program at the Massachusetts General Hospital using DOT for non-invasive, portable functional brain monitoring in neurological patients. A study is under way to monitor stroke progression and to apply DOT as a means to monitor treatment for rehabilitation. Drs. Sutton and Strangman also have plans for DOT applications in neuroscience and studies of cognitive dysfunction—in particular, for the monitoring and evaluation of rehabilitation from stroke or brain injury. Other medical applications for DOT are being pursued at several centers across the country. More generally, Earth-based applications of the refined DOT and AIMS technologies are numerous. Examples (not comprehensive) appear below:

- Functional brain imaging of (or even during) rehabilitation from stroke or brain injury
- Cerebral imaging or monitoring in remote settings (rural health centers, mountainside, etc.)
- Detection of tissue ischemia (esp. muscle, abdominal, brain)
- Pediatric brain imaging/evaluation (particularly for young, highly-animated patients)
- Stroke detection and monitoring
- Breast cancer detection
- Autonomous medical diagnosis in remote (on-location) settings

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	James D. Thomas, M.D.
ORGANIZATION:	The Cleveland Clinic Foundation
PROJECT TITLE:	Diagnostic Three-Dimensional Ultrasonography: Development of Novel Compression, Segmentation and Registration Techniques for Manned Space Flight Applications

Project Executive Summary

The NSBRI has identified that the efficient and automated delivery of health care in space is a key research arena for the future. Specifically, they propose to develop a "Smart Medical System" that will be able to monitor crew health, identify deviations from ground-based norms, and allow timely intervention by crew members who may have only a moderate amount of training in medicine. For the last three years, the principal investigator and colleagues have worked closely with NASA scientists, flight surgeons, and engineers to optimize research and diagnostic ultrasound aboard the International Space Station (ISS) and thus are well positioned to develop the necessary tools and techniques to integrate ultrasound into the Smart Medical System. A principal limitation of ultrasound technology is its extreme dependence on the expertise of both the acquiring examiner and the interpreting physician. This is particularly true of two-dimension ultrasound, where the examiner is required to obtain precisely oriented anatomical sections of the organ of interest.

Three-dimensional ultrasound has the advantage of acquiring a large anatomic volume from a single ultrasonic window, and thus may be less dependent upon the expertise of the examiner. Furthermore, this large volume may contain sufficient anatomic landmarks to allow unambiguous registration with previously obtained three-dimension data from either ultrasound or other modalities such as magnetic resonance imaging (MRI) or computed tomography (CT). One could thus envision a system by which whole organs or even the entire body would be imaged in three-dimensions prior to launch; data which could be used to compare with subsequently obtained three-dimensional data sets using in-flight ultrasonography. The overall purpose of this grant is therefore to perform ground-based research, development, and validation aimed at optimizing diagnostic ultrasound in manned space flight, with the following general hypothesis:

Unifying hypothesis: Serial three-dimensional ultrasound examinations will enhance diagnostic capabilities in manned space flight.

The technical aspects of this program will be pursued with the following specific aims:

1. Optimize the acquisition methods for three-dimensional sonography, utilizing reconstruction and real-time techniques.
2. Develop techniques for registering anatomical images from two- and three-dimensional ultrasound with those obtained from prior ultrasound examination and from magnetic resonance and computed tomographic imaging, considered "gold standards" for non-invasive anatomical imaging.
3. Develop tools for abstracting, in an automated fashion, anatomical changes from serial three-dimension and two-dimension ultrasound studies.
4. Develop algorithms for the optimal compression of three-dimensional ultrasound images and refine current two-dimensional compression algorithms.
5. Assess the ability of novice examiners to obtain three-dimensional sonographic data sets following minimal training.

These objectives will be pursued using data from a variety of *in vitro*, animal and clinical models. In particular, we will take advantage of a well-established collaboration with the National Institutes of Health, which permits highly sophisticated chronic animal models to be examined with a minimum of additional resources. Although the tools developed here should be applicable to any organ of the body, we will focus our efforts on the kidneys and the heart.

At the conclusion of this project, we anticipate delivering to the NSBRI and its Smart Medical System a set of algorithms and software for the non-rigid morphological registration and comparison of serial two- and three-dimensional ultrasound data sets and validated algorithms for optimal compression of four-dimensional ultrasound data. In addition to these technical deliverables, our validation work on nephrolithiasis will provide important diagnostic clues for assessing this condition in manned space flight. Similarly, the work on cardiac mass regression following unloading will be invaluable to the NASA research and medical operations community in assessing the impact of long-term space flight on cardiac atrophy and utility of prophylactic countermeasures.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	James D. Thomas, M.D.
ORGANIZATION:	The Cleveland Clinic Foundation
PROJECT TITLE:	Echocardiographic Assessment of Cardiovascular Adaptation and Countermeasures in Microgravity
END DATE:	07/31/2004

Project Executive Summary

The cardiovascular system undergoes significant changes in microgravity, including an early cephalad shift of lower extremity blood volume, loss of plasma volume over 24 to 48 hours, and long-term reduction in ventricular chamber volume and mass. In the weightless environment, these alterations generally are well tolerated, but upon return to Earth, astronauts often suffer from serious orthostatic intolerance and reduced exercise capacity, changes that may limit the long-term presence of man in space. It is essential that the mechanisms for these alterations be understood so that reliable countermeasures can be tested and implemented. Hypovolemia, cardiac atrophy, and autonomic dysfunction have each been hypothesized to contribute to this post-flight debility, but their relative importance is unclear. Furthermore, it is unknown whether actual abnormalities in the myocardium itself develop with long-term space flight. Therefore, reliable portable noninvasive methods will be needed in order to detect and quantify these changes.

Alone among such imaging modalities of radiography, magnetic resonance imaging and computerized tomography, echocardiography has the unique ability to characterize cardiovascular anatomy and physiology in ground-based models, pre- and post-flight, and most importantly during flight. Indeed, the Science Working Group (SWG) for the International Space Station (ISS) Human Research Facility (HRF) has recognized the primacy of ultrasound for medical diagnosis and physiology research, with plans to launch a specially modified commercial ultrasound instrument to the ISS in 2001. Echocardiography is similarly being used before and after shuttle flights and in a variety of bed-rest studies sponsored by NSBRI and NASA. Unfortunately, while ultrasound has the potential for high spatial and temporal resolution imaging of the heart, in the past it has been severely limited by operator inexperience and inconsistency in its subjective interpretation. Needed are new methodologies for assessing the load-independent function of the heart and consistent, objective quantification of a wide range of NASA echo studies, whether obtained on the ground, in flight or in experimental models. We propose to provide such a facility while validating novel methods for the load independent assessment of myocardial function. Our central hypothesis is that:

Microgravity affects cardiovascular function not only through changes in chamber volume and mass but also through changes in myocardial properties.

A definitive test of this hypothesis is at least several years away when dedicated life science missions are possible aboard the ISS. However, within the scope of this grant, we propose several specific aims that will be critical to the ultimate comprehensive study of the cardiovascular system in space. Key issues: 1) Validation of non-invasive Doppler echocardiographic indices for the assessment of left ventricular contractility and relaxation including color M-mode Doppler derived diastolic intraventricular pressure gradients (IVPG) and tissue Doppler derived myocardial systolic and diastolic strain rates (e's, e'd); 2) Validation of Doppler derived exercise cardiac output and contractile reserve and their potential utility for the early detection of myocardial dysfunction during prolonged space flight. Additional deliverables to NSBRI: 3) Development and distribution of stand-alone software and algorithms

for implementing the quantitative analysis of Doppler echocardiographic data, as described above, so they may be applied to ultrasound data obtained from remote sources; 4) Establishment of an Echocardiographic Core Facility to the NASA research and clinical community, capable of applying standard and novel analysis techniques in a rigorous fashion to echocardiographic data obtained from selected ground-based experimental models, pre- and post-flight examinations, and eventually from in-flight acquisitions.

If successfully implemented, these aims will allow the cardiovascular sequelae of space flight to be studied much more rigorously, while providing consistent, objective echocardiographic interpretation to the entire NASA community.

RESEARCH AREA:	Smart Medical Systems
PRINCIPAL INVESTIGATOR:	James D. Thomas, M.D.
ORGANIZATION:	The Cleveland Clinic
PROJECT TITLE:	Echocardiographic Assessment of Cardiovascular Adaptation and Countermeasures in Microgravity

Project Executive Summary

Among the most serious of the risks identified by NASA in the area of cardiovascular alterations are serious dysrhythmias and the development of orthostatic intolerance. Prolonged exposure to microgravity may lead to a reduction in cardiac performance, particularly during times of stress and that undiagnosed cardiovascular disease may manifest during long missions. The PI and colleagues have worked closely with NASA and NSBRI over the last six years to optimize use of ultrasound in the space program as an investigative modality, addressing fundamental cardiovascular problems in need of countermeasures development. We propose the following specific aims:

- 1) Extension of work to calculate two-dimensional myocardial strain, improving sensitivity for detecting preclinical alterations in cardiac function.
- 2) Since early cardiac disease is usually manifest initially during exercise stress, we will develop and validate the tools to apply 2-D strain in graded exercise to detect myocardial dysfunction in its earliest phases, allowing both diagnostic capabilities and a means of judging exercise as a countermeasure.
- 3) To continue our ongoing study of the magnitude and predictors of left ventricular mass regression following acute volume and pressure unloading as a ground-based analog for manned space flight. This work will continue to focus on patients undergoing aortic valve surgery, but exploit recent knowledge of the roles of cytokines and integrins involved in cardiac hypertrophy and regression as well as emerging technologies such as gene chip analysis.
- 4) To develop, in collaboration with OBPR Fundamental Physics scientists from Glenn, a sophisticated fluid-structure model of the left ventricle constrained by the pericardium to investigate the impact that microgravity has on unloading the heart by a removal of pericardial constraint.

This work will be closely focused on risks and critical questions identified by the Cardiovascular Alterations Team as described in the Bioastronautics Critical Path Road Map Baseline Document. If successful, this project will enhance assessment of cardiac function during long-duration missions and potentially suggest cytokine promoters or signal transduction pathways that could be targeted for cardiac atrophy countermeasures. In addition, we will continue to provide the facilities of our core laboratory for access by investigators throughout the NASA and NSBRI programs in need of assistance in acquiring or analyzing ultrasonic data.

NSBRI RESEARCH PROGRAM TECHNOLOGY DEVELOPMENT

Team Leader: **Buckey, J. C.** **Dartmouth**

**Associate Team
Leader:** **Qin, Y.** **SUNY**

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RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Isaac N. Bankman, Ph.D.
ORGANIZATION:	Johns Hopkins University Applied Physics Laboratory
PROJECT TITLE:	Development of a Space Qualifiable MRI System

Project Executive Summary

This project is to develop a proof-of-concept engineering model of a space qualified Magnetic Resonance Imaging (MRI) system for small animals and astronaut limbs with mass of < 130 kg and average power when on but not scanning < 1 kW and when scanning < 1.2 kW, not including the processor. An onboard processor or a high-performance PC can be adapted. MRI provides high-resolution, high-quality anatomical information without ionizing radiation so it can be safely used repeatedly to track changes without deleterious effects. As a result, the study of physiological alterations in space and the development, verification, and maintenance of countermeasures will be significantly enhanced.

Mice and small rat models are useful surrogates to carry out in-orbit physiological studies. Measuring alterations in the limbs of astronauts, especially the lower limbs, will provide partial confirmation of the effectiveness of proposed countermeasures and the utility of Earth-based animal models. In-flight MR imaging of mice and rats will especially benefit the countermeasure developments of several of the NSBRI research teams.

The proposed concept is based on traditional MRI principles and uses advanced technology and advanced engineering techniques to reduce mass and power to acceptable levels. The system consists of a 1 to 1.5 Tesla cryogen-free high temperature superconducting magnet subsystem and advanced electronics that will have magnetic field inhomogeneities ≤ 8 ppm over a spherical imaging volume of 10 cm diameter and ≤ 10 ppm out to 15 cm diameter. The magnet cryocooler subsystem will be designed using high temperature superconducting materials to significantly reduce the mass and power of the cryocooler.

The highest resolution mode gives a resolution of 117 microns for small animals over a spherical imaging volume of 6 cm diameter and a resolution of 352 microns for human limbs over a spherical imaging volume of 18 cm diameter. The standard resolution mode will provide a resolution of 234 microns and 703 microns, respectively. The pulse sequence scenarios used will be those traditionally used in MR imaging to achieve images that are proton-density, T1 or T2 weighted, so that a significant amount of structural information will be available. Because of budget limitations, only selected electronics will be reengineered to demonstrate the minimum mass and power that can be achieved.

The team is composed of individuals and organizations with a unique combination of expertise including: MRI systems development at the General Electric Research and Development Center, advanced MRI development and small animal experimentation at the Johns Hopkins School of Medicine, and the development of reliable medical and low-mass, low-power systems for space applications at the Johns Hopkins University Applied Physics Laboratory.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Jay C. Buckey, M.D.
ORGANIZATION:	Dartmouth Hitchcock Medical Center
PROJECT TITLE:	Improved Bubble Detection for EVA
END DATE:	06/30/2004

Project Executive Summary

The objective of this project is to improve EVA efficiency and safety through the *in-vivo* validation of a unique ultrasonic bubble-sizing and detection instrument. This instrument exploits bubble resonance by using two frequencies of ultrasound (dual-frequency ultrasound) to detect and size bubbles in tissue and blood. The original aims of the project were to: (a) establish the appropriate transducer configurations, electronic settings and instrument enhancements to detect and size bubbles reliably *in-vivo*, (b) compare the new bubble monitoring technique to Doppler, and use it to investigate decompression sickness and (c) develop the capability to size small bubbles in tissue. The progress toward each of these aims is summarized below.

Progress

Progress toward establishing the appropriate transducer configurations, electronic settings and instrument enhancements to detect and size bubbles reliably *in vivo* – This aim has been accomplished. This was done using a stepwise approach. First, experiments were performed *in-vivo* using agitated saline (the agitated saline contains small bubbles). The transducers of the dual frequency device were aimed into the right ventricle. Agitated saline was injected intravenously while data were collected with the device. These experiments demonstrated that bubbles could be detected as they move through the right ventricle and right atrium. These experiments established the technical knowledge (transducer position relative to anatomical features, equipment settings, etc.) needed to monitor bubbles during subsequent decompression experiments.

In the decompression experiments, the transducers were positioned on the chest wall and the ultrasonic energy was beamed into the right ventricle. The pump frequency (which selects the size of bubble that will be imaged) was increased stepwise from 30 kHz to 180 kHz in 5 kHz increments. At each frequency data were taken with the pump transducer on and off. By comparing the signals returned with the pump on to that with the pump off for each pump frequency, a histogram of bubble sizes could be produced. This work is significant, since the ability to produce bubble size histograms during decompression stress is a new capability that may have both operational and research uses.

Progress toward comparing the new bubble monitoring technique to Doppler, and using it to investigate decompression sickness – This aim has been advanced by comparing the signals obtained with the dual frequency device to a standard clinical ultrasound instrument. Early indications are that the dual frequency device may detect bubbles prior to Doppler, but work in this area is ongoing. A Doppler capability has also been added to the dual frequency device, primarily to assist in aiming the transducers at the ventricle but also as a complementary means to signify the presence of bubbles.

The combination of the Doppler and dual frequency ultrasound is being used to: (a) evaluate the changes in bubble size during the evolution of decompression sickness and (b) evaluate perfluorocarbons as a potential treatment for decompression sickness. The combination of the

two bubble detection capabilities into one device provides a versatile instrument for studying decompression sickness.

Progress toward developing the capability to size small bubbles in tissue – This aim has been advanced through a variety of *in vitro* and *in vivo* studies. The tissue bubble detection effort has two goals: (a) to evaluate whether very small bubbles (< 30 microns) can be detected in tissue, since decompression sickness theory suggests that small bubbles may exist in tissue normally at ambient pressure and (b) to detect larger bubbles in tissue and in the vasculature that may cause symptoms during decompression sickness. Current efforts have focused on the first goal. Early studies demonstrated signals consistent with bubbles in the thigh of the swine. These signals were found only at particular locations. In the original implementation of the detector, however, the source of the mixing signals could not be determined because of poor spatial resolution. The modified bubble detector now allows for sampling at selected depths, so the mixing signals can be correlated with anatomic structures. This has shown that mixing signals are most likely returned from interfascial planes, i.e. the areas between muscle groups.

Validating tissue bubble detection requires a reliable way to demonstrate that signals detected in tissue originate from bubbles. Current research is focused on developing *in-vitro* tissue bubble simulators capable of generating prototypical small bubbles to test the tissue bubble detection equipment. Several *in-vitro* methods are under evaluation, including contrast agent embedded in gelatin, decompressed gelatin, and schemes involving the passage of high-pressure air through very small pipettes.

The tissue bubble detection work is significant since the ability to detect and size bubbles in tissue would be a new and unique capability.

Plan for the Coming Year

In the coming year the plans are to:

- Refine the bubble size histogram capability;
- Use histograms to evaluate bubble sizes during decompression stress and during interventions to treat decompression sickness (e.g. administration of perfluorocarbons);
- Advance tissue bubble detection; and
- Pursue human use approval for the device.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Jay C. Buckey, Jr., M.D.
ORGANIZATION:	Dartmouth College
PROJECT TITLE:	Improved Bubble Detection for EVA

Project Executive Summary

Assembly of the International Space Station and future space exploration require extensive and unprecedented extra-vehicular activity. Current spacecraft and suit designs force astronauts to move between different pressure environments, making decompression sickness (DCS) a potential risk. DCS risk mitigation strategies reduce operational efficiency. The objective of this effort is to improve EVA efficiency and safety by developing and validating new bubble-detection technology using dual-frequency ultrasound. The Creare dual-frequency instrument (CDFI) can detect and size bubbles through the chest wall as they move through the heart. Also, signals consistent with bubbles can be detected in tissue. Potentially, this technology could be used to: (a) characterize bubble dynamics during decompression sickness (DCS), (b) detect the earliest stages of DCS, (c) develop and evaluate non-compressive countermeasures for DCS, (d) diagnose DCS in tissue or joints, and (4) mitigate DCS risk by improving preventive strategies such as oxygen pre-breathing and limiting activity at particular times.

Detecting and sizing bubbles intravascularly (a new and unique capability) allows for bubble size histograms to be constructed during the development and treatment of DCS. The change of bubble size distribution during decompression stress may indicate the progression of DCS. Preliminary data indicate the CDFI may identify bubbles earlier than current Doppler or imaging ultrasound techniques. One goal of this project is to demonstrate the capabilities of the CDFI in DCS. Experiments using anesthetized swine after decompression will be performed to test the CDFI. An accurate and reliable way to assess intravascular bubbles may offer a way to evaluate non-compressive therapies, such as perfluorocarbons, for DCS. Studies on the effect of perfluorocarbons on bubble size and frequency during DCS will be performed in swine exposed to decompression stress.

Tissue bubble detection is also a unique capability. The CDFI can potentially detect very small bubbles (the possible precursors of larger bubbles in tissue or blood) and identify larger bubbles in areas with symptoms of pain or discomfort consistent with DCS. A goal of this project is to validate tissue bubble detection for both very small (<10 micron) and large (50 micron) bubbles. *In-vitro* tests and studies using swine exposed to compression and decompression will be performed to validate the CDFI in tissue.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Harry K. Charles, Jr., Ph.D.
ORGANIZATION:	Johns Hopkins University Applied Physics Laboratory
PROJECT TITLE:	AMPDXA Scanner for Precision Bone and Muscle Loss Measurements During Long-Term Space Flight
END DATE:	03/31/2004

Project Executive Summary

The purpose of the Advanced Multiple Projection Dual Energy X-ray Absorptiometry (AMPDXA) Scanning System project is to design, build, and test a precision scanner system for monitoring the deleterious effects of weightlessness on the human musculoskeletal system during prolonged spaceflight. The instrument uses dual energy X-ray absorptiometry (DXA) principles and is designed to measure bone mineral density (BMD), decompose soft tissue into fat and muscle, and derive structural properties (cross-sections, moments of inertia). Such data permits assessment of microgravity effects on bone and muscle and the associated fracture risk upon returning to planetary gravity levels. Multiple projections, coupled with axial translation, provide three-dimensional geometric properties suitable for accurate structural analysis. This structural analysis, coupled with bone models and estimated loads, defines the fracture risk. The scanner will be designed to minimize volume and mass (46 kg goal), while maintaining the required mechanical stability for high-precision measurement. The AMPDXA will be able to detect one percent changes in bone mass and geometry and five percent changes in muscle mass.

The AMPDXA project is a joint effort between the NSBRI's Technology Development Team, the Bone Demineralization/Calcium Metabolism Team, and the Muscle Alterations and Atrophy Team. Its goal is to provide the high precision monitoring system necessary to fully assess both the deleterious effects of weightlessness on the bones and muscles and the effectiveness of any countermeasures. We believe that any pharmacological or exercise-related countermeasures used by astronauts to mitigate microgravity effects will require efficient and timely monitoring. Moreover, the monitoring device must be capable of being used by astronauts during spaceflight so that feedback can be dynamically employed to regulate countermeasure doses. The system design will be such that intelligent, but not necessarily medically trained, personnel will be able to create scans that will provide all of the accuracy and precision necessary. Readouts and displays for the AMPDXA instrumentation will be specifically designed to provide useful (real-time) feedback information to both the astronauts and the ground-based physician monitoring team (as permitted by the mission dynamics).

Current bone and muscle mass measurements (via conventional DXA or ultrasound) are regional averages that obscure structural details. Since the mechanical consequences of lost bone and muscle are reflected in the structure, an absolute determination of skeletal mechanical competence is needed to supplement the loss measurements. Engineering properties of the bones can be derived from DXA-generated BMD data. Our method derives geometrical measurements from the BMD images. From such images, we extract BMD profiles at important skeletal locations (e.g., proximal shaft and femoral neck). Key properties measured and derived from these profiles include the BMD, the subperiosteal width, the section modulus (related to strength), and the cortical dimensions.

Under the original proposal effort, FY 1998-2000, the AMPDXA project made significant progress in several key areas: (1) instrument development, (2) algorithm development for BMD

image extraction and structural analysis, and (3) bone reconstruction and modeling techniques. During the FY 1998-2001 period, both a full-sized (one-meter source-to-detector distance) Laboratory Test Bed and a system for human testing were constructed. This system was initially called the Clinical Test System in previous reports, but is now called the Human Test Bed to better reflect the nature of the human testing to be performed on the system. The Laboratory Test Bed was utilized to verify principles and theoretical predictions and demonstrate that the AMPDXA techniques worked and produced results with the expected precision. The Human Test Bed has even greater precision.

The Human Test Bed incorporates high-precision, rotational and translational stages to provide the scanning capability to carry out qualification tests on human subjects. Since the Human Test Bed is designed to operate only on Earth, the table, gantry, and associated equipment were not built to the size and mass requirements of an AMPDXA unit for spaceflight. In fact, the unit was built on a used CT scanner. Employing used equipment for some of the structural elements and rotating parts and machinery allowed critical resources to be focused on the information extraction and analysis issues leading to human testing.

The image extraction capability of the AMPDXA is not only the BMD image higher resolution, but also the mass distribution in a projected thickness of a femur slice contains much more structural detail than conventional DXAs. The high frequency content of the BMD spatial projections are reproducible and provide information on the bone's microstructure. Using multiple projections (three or greater) about the bone axis allows structural properties (e.g., bending strength) to be obtained independent of patient position. Initial experimental measurements with different sets of three projections showed that the principal moments of inertia could be determined within three to four percent. Additional projections (above three) reduce this number further. Our original experimental system also had some known non-linearities, which have since been removed, and our error in the three-projection estimation of moments is less than one percent.

For the 2002 period, we are focused on resolving certain key issues about the AMPDXA and then successfully using the AMPDXA for human testing. These key issues include: (1) unequivocal demonstration that multiple projection technology improves BMD accuracy and collects structural details, (2) the structural details can be converted into bone reconstruction models that preserve mechanical behavior, (3) the reconstruction models can be utilized to predict risk of fracture, (4) soft tissue can effectively be distinguished from bone and decomposed into fat and muscle, (5) data can be collected reliably and repeatedly on human subjects using the Human Test Bed, and (6) the Human Test Bed can be utilized in research studies on bone and muscle loss.

A dual monitor computer system currently operates the AMPDXA as well as records and displays image maps (bone mineral density, muscle, etc.) at near real-time speeds. The main screen presents two views of the BMD images of a human hip collected from a live human test subject. The major accomplishments during the period include reverification and calibration of the Human Test Bed after the move, improving the AMPDXA operational software (providing full documentation and configuration control), refining the image extraction algorithms, demonstrating the Human Test Bed's accuracy and repeatability, and human imaging. Approval for our human testing protocol has been granted by the Institutional Review Board at the Johns Hopkins Medical Institutions.

The AMPDXA project has many implications for future research and development. The AMPDXA, as described above, has direct application to risk reduction in NASA's Critical Research Path. The AMPDXA is capable of real-time monitoring of bone and muscle loss at extremely high precision. Since the results are patient-specific and not tied to volumetric averages and statistical norms, the AMPDXA is a very useful tool for monitoring the effectiveness of countermeasures as well as determining risk of fracture under various loading conditions and activity scenarios. The AMPDXA also appears to be a natural adjunct to Earth-bound research on the effects of aging and disuse on bone integrity. It could also be used as a routine screening tool for osteoporosis and as a monitoring instrument for osteoporosis drug therapy.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Harry K. Charles, Jr., Ph.D.
ORGANIZATION:	Johns Hopkins University Applied Physics Laboratory
PROJECT TITLE:	Ground-Based Measurement of Bone Loss in Astronauts Using AMPDXA Ground-Based Clinical System

Project Executive Summary

The Johns Hopkins University Applied Physics Laboratory and the School of Medicine are building a Ground-Based Clinical System (GCS) using Advanced Multiple Projection Dual-Energy X-ray Absorptiometry (AMPDXA) principles to go beyond bone densitometry to measure the structural mechanics of bones and muscles. Exposure to microgravity during long-duration space flight decreases bone and muscle mass, but the implications for injury differ from similar losses on Earth and cannot be discerned by densitometry. By providing a high precision means for scanning astronauts before and after space flights, the GCS will be able to monitor musculoskeletal strength and the effectiveness of countermeasures.

The GCS could have a much larger impact on clinical medicine. Millions of people suffer from osteoporosis and other disorders that degrade bone mechanical strength. Research and clinical medicine will benefit from a more definitive method for diagnosing bone disease, monitoring its progress and assessing the efficacy of treatments.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Brian L. Davis, Ph.D.
ORGANIZATION:	The Cleveland Clinic Foundation
PROJECT TITLE:	Design and Validation of a Dynamic Exercise Countermeasure Device
END DATE:	06/30/2004

Project Executive Summary

Exercise in space is a promising countermeasure to space flight-induced bone loss and muscle atrophy. The objective of this study was to develop a dynamic exercise countermeasure device that permits a jumping exercise in space. The device benefits multiple physiological systems - muscular, skeletal, neurovestibular and cardiovascular - without transmitting vibrations and impact forces to other parts of the spacecraft.

This three-year project was divided into three phases. In Year 1 we collaborated with Foster Miller Inc., a company that has expertise in the design of both lightweight structures and vibration isolation methodology, to construct a device that permits dynamic jumping exercise in microgravity and that is suitable for the International Space Station.

In Year 2 we conducted ground-based studies in the exercise device to verify that muscle activation patterns are not compromised by the exercise device and that limb loading data, as measured by forces experienced under the feet during jumping, are in the range that is believed to maintain the integrity of bone. Four different exercises were performed in the exercise device: 1) standard toe raises, 2) standard "squats", 3) "jumping rope" - in which consecutive mini-jumps were performed, and 4) maximum jumps - in which a series of maximum "vertical" jumps were performed. Test results are very promising in that we have been able to demonstrate that subjects exercising in the DECD experienced ground reaction forces ranging from 2-3 times bodyweight. These forces are greater than what is currently elicited by astronauts exercising in space and jumping exercises may, therefore, prove to be a more effective countermeasure to spaceflight-induced deconditioning.

In Year 3 we confirmed the efficacy of the exercise device in true microgravity through experiments on the KC-135. Ground reaction forces were found to be at levels of high enough impact to provide an adequate daily loading stimulus to alleviate bone loss. With regards to muscle activity, results suggest that exercises in the DECD will stimulate higher muscular force requirements of certain muscle groups to better maintain overall muscle mass of the legs.

Earth-Based Applications of Research Project

The benefits of exercising the cardiovascular and musculoskeletal systems are widely accepted on Earth. Bone demineralization (bone mass loss) is a well-documented physiologic effect of long-duration spaceflight and as a consequence of disease and aging. Animal experiments on Earth have clearly indicated that: (1) certain bone strains and strain rates stimulate bone deposition, and (2) repetitive loading of the lower extremity can increase osteonal bone formation even as proximally as the vertebral column.

Such studies have also indicated that a relatively small number of appropriate weight-loading cycles may be sufficient to stimulate bone deposition. By extending the philosophy of designing exercise modalities that elicit high impact forces, a dynamic exercise countermeasure device (DECD) that utilizes jumping as the mode of exercise will benefit a large population on Earth. This includes developing exercise protocols for patients with osteoporosis. Many such exercise plans are used; however, jumping may provide a more efficient and less time consuming way to address issues decreasing bone loss.

An added benefit of the DECD is that it may aid patients with musculoskeletal disorders or traumatic injuries, which require rehabilitation therapy to strength and increase muscle mass to return to full function. This is due in part to substantial activity found in lower extremity muscles during the resistive exercise such as squats and toe raises. In this manner, the DECD could act in the same manner as gym/therapy equipment.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Richard H. Maurer, Ph.D.
ORGANIZATION:	Johns Hopkins University Applied Physics Laboratory
PROJECT TITLE:	Neutron Energy Spectrometer Flight Experiments
END DATE:	03/31/2004

Project Executive Summary

High-energy charged particles of extra-galactic, galactic and solar origin collide with spacecraft structures in Earth orbit outside the atmosphere and in interplanetary travel beyond the Earth's magnetosphere. These primaries create a number of secondary particles inside the structures that can produce a significant ionizing radiation environment. This radiation is a threat to long term inhabitants for space missions and produces an increased risk of cancer, CNS and DNA damage.

The primary high energy cosmic rays and trapped protons collide with spacecraft materials such as aluminum and silicon and create secondary particles inside structures that are charged particle fragments and neutrons. The effect of tens of grams per square centimeter of structure or atmosphere is to convert and multiply the primary proton "beam" into a secondary environment dominated by neutrons. Charged protons are readily detected and instruments are already in existence for this task.

Neutrons are electrically neutral and much more difficult detect. These neutrons are estimated to contribute 10-30% of the dose inside space structures and cannot be ignored. Currently there is no compact, portable and real time neutron detector instrumentation available for use inside spacecraft or on planetary surfaces.

As a product of our previous NSBRI funding, we had met the original aims of the project to design and fabricate an engineering prototype neutron spectrometer that was flown on F-15 and F-18 aircraft flights from NASA Dryden Flight Center. The spectrometer consists of both low and high energy subsystems. The detection of low energy neutrons (0.025 eV-1 MeV) is accomplished using a conventional helium 3 gas tube. The detection of high energy neutrons (5-800 MeV) is achieved using a 5 mm thick lithium drifted silicon solid state device.

The neutron spectrometer was flown on two flights 13-14 August 2001 in a pod under the wing of an F-18. A third successful flight in the same pod under the fuselage of an F-15 was executed in October 2001. The main positive result from the three flights was the verification of our engineering design and not the limited data obtained due to the short duration (~2 hours) of the aircraft flights. The value for our hardware was the proven approach in handling high voltage at high altitude corona region that will be employed for balloon flights.

Efforts in FY 2002 and 2003 were directed at designing and fabricating a neutron spectrometer for high altitude balloon flights. The electronics were made more robust and compact for the balloon flight instrument. The detector suite was changed to include a Medium Energy Spectrometer (MES) for the fast neutrons in the 1-20 MeV energy range in addition to the thick silicon detector for the >20 MeV neutron energies. The helium 3 tube was not included for the low energy neutrons (10 keV-1 MeV) since this system was validated on the aircraft flights and

will be readily available for flight efforts. The MES is a Bicron 454 plastic scintillator detector system that borrows from the development of a similar system for the APL unmanned MESSENGER mission to Mercury.

Sophisticated energy deposition signal time discrimination allows the observation of both scattering and capture peaks of the neutrons in the Bicron detector for individual counts and energies. Development of experience in the calibration and use of this detector system was one of the interim goals of the project. Balloon flights were executed from Fort Sumner, NM at an altitude of 85,000 feet on two occasions--October 9, 2002 and October 9, 2003. The altitude of 85,000 feet was chosen since the amount of atmosphere remaining (~20 grams per square centimeter) is the same as the amount of carbon dioxide at the surface of Mars and should yield a reasonable simulation of the downward neutron spectrum on Mars. The October 2002 flight did not yield any useful scientific data due to engineering problems with the high voltage connection to the silicon detector and ground loop issues between the electronics and the aluminum container of the instrument. The problems were corrected during FY 03, and the October 2003 flight yielded useful scientific data.

Initial analysis showed that we obtained a highly moderated neutron energy spectrum with the majority of neutrons in the energy range between 20 and 35 MeV. Modeling of the detector shielding geometry is necessary to deduce that on the lid of the instrument relative to that measured at the detector location. This task is our starting point in FY 05.

For the evaluation of spacecraft structural and shielding materials, we built a stack detector version of the neutron spectrometer compatible with ground-based accelerator research. We verified its successful operation at Columbia University's RARAF in November 2001, and then proceeded with spacecraft shielding experiments using 200 MeV proton beams at the Indiana University Cyclotron Facility in November 2002 and November 2003 and 500 MeV proton beam at TRIUMF in Vancouver, Canada in September 2003. Aluminum, carbon and polyethylene block targets were used to simulate spacecraft materials. The results yielded neutron production energy spectra showing the reduced yield from carbon-based materials and the moderating (scattering) effects of polyethylene when compared to aluminum. Our data validate the recent conclusion about aluminum being the least suitable spacecraft material with respect to increased neutron production and human radiation effects. This was the last year for this project.

Earth-Based Applications of Research Project

The main Earth-based application for this project/instrument development is related to homeland security and the monitoring of freight/cargo for the presence of fast and high energy neutrons from clandestine radioactive material.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Richard H. Maurer, Ph.D.
ORGANIZATION:	Johns Hopkins University Applied Physics Laboratory
PROJECT TITLE:	Combined Ion and Neutron Spectrometer for Space Applications

Project Executive Summary

We propose to design and fabricate a prototype Combined Ion and Neutron Spectrometer (CINS) for space applications. This instrument will improve upon existing charged particle species and energy spectrometers (e.g., MARIE on the Mars Odyssey mission) and incorporate the capability of the previously developed NSBRI Neutron Energy Spectrometer to yield a single and complete ionizing radiation environment monitor for application in space habitats and transport vehicles. An improved charged particle/ion telescope detector system will be designed at Lawrence Berkeley Laboratory. It will be combined with the NSBRI Neutron Spectrometer at The Johns Hopkins University Applied Physics Laboratory into a single instrument for ground-based research at accelerators by using multiple fast channels of analog and digital electronics based on JHU/APL's experience with NEAR and MESSENGER spacecraft instruments. This approach merges the sophistication of space flight design with the low cost of non-flight electronic components.

After fabrication and calibration, CINS will be used to compare its energy spectra with the Tissue Equivalent Proportional Counter (TEPC) to investigate basic hypotheses about 1) the lack of radiation environment information from the TEPC Linear Energy Transfer spectrometer due to detection limitations with respect to high energy protons, low energy heavy ions and almost all neutrons; and 2) the increased risk due to the multiplication of primary beams of protons and heavy ions by spacecraft materials which produces a secondary particle environment of protons, neutrons and heavy ion fragments. The significant experience of the CINS research team with instrument hardware, software and complex data analysis techniques promises that CINS will be the ultimate radiation environment monitor for manned space applications.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Vincent L. Pisacane, Ph.D.
ORGANIZATION:	United States Naval Academy
PROJECT TITLE:	Microdosimeter Instrument System Suitable for Space Flight

Project Executive Summary

The objective of this project is to develop a rugged, portable, low power, lightweight, solid-state radiation instrument MIDN (MicroDosimeter iNstrument) to measure probability or frequency distributions of energy deposited in real time in cell-size structures from charged and neutral primary and secondary radiations. Assessment of the space radiation environment addresses the development of a countermeasure of the highest priority for space exploration. Determination of the magnitude of the average radiation quality, Qave, is necessary to calculate the dose equivalent, which is assumed to be proportional to risk and upon which regulatory limits in space are based. Microdosimetry spectra are the most accepted data for calculating Qave. Consequently, the observations are more reliable as a monitor of human cell damage than other macroscopic detectors that measure fluence.

Hypotheses:

1. A small, compact, and portable, flight qualifiable, solid-state microdosimeter can be developed to measure quantitative information on the dose and dose distribution of energy deposited in silicon cells of tissue size and by inference in tissue.
2. Analysis of MIDN data from radiation beam experiments and comparison with radiation transport codes can provide quantitative information on the radiation environment, potential risk, and the accuracy of the codes to correctly calculate energy-deposition spectra.
3. MIDN data from radiation beam experiments correlated with radiation transport codes can determine the effectiveness of selected materials to minimize the total risk from primary and secondary radiation.

MIDN countermeasure capabilities are to:

1. Make real-time measurement of radiation environment to assess and reduce risk (dose equivalent).
2. Actively warn crew during onset of enhanced radiation.
3. Allow crew to determine safe locations during enhanced radiation.
4. Provide observations to validate and improve models for the space radiation environment, the effectiveness of shielding materials, and use with a human phantom could provide microdosimetric information for different organs or tissue types (for example bone versus muscle).

A preliminary laboratory breadboard, partially funded by NASA, has demonstrated feasibility. Work on this project will increase the instrument's CRL from 5 to 7.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Richard S. Potember, Ph.D.
ORGANIZATION:	Johns Hopkins University Applied Physics Laboratory
PROJECT TITLE:	Real-Time Analysis of Biomarkers and Countermeasures Using a Miniature Time-of-Flight Mass Spectrometer
END DATE:	09/30/2004

Project Executive Summary

Original Aims of the Project

- To design, develop and test a fast, portable gas chromatograph – time-of-flight mass spectrometry (GC-MS) system for future human spaceflight applications. It will provide complementary information to the MALDI method.
- To demonstrate that the miniature TOF system is capable of detecting and quantifying different biomarkers that appear in serum or urine during space flight. Detection and quantification of critical biomarkers using the miniature TOF technology will allow real-time monitoring of damage on-orbit, and the mass spectrometer can also be used to study the effectiveness of countermeasures in spaceflight. The results of this effort should be comparable to measurements made in a clinical laboratory facility using established assays.
- To validate that the miniature time-of-flight mass spectrometer is an important diagnostic tool that can be applied to measure important bone biomarkers and the effectiveness of applied countermeasures in human urine and serum samples.
- To compare standard methods of hormone analyses for melatonin and cortisol to that of the Miniature Mass Spectrometer. The development of online methods for monitoring and assessing the status of circadian organization is listed as one of the five primary themes for the Human Performance Factors, Sleep and Chronobiology Team.
- To develop sampling and sample preparation techniques that enable the MALDI TOF mass spectrometer system to reliably detect, identify and quantify extremely low levels of chemical and biological substances in complex body fluids with very low error rates.

Key Findings

We have designed and built a new miniature - mass spectrometry (GC-MS) system with a 3-inch analyzer for human spaceflight applications. This miniature instrument will provide new capabilities in the area of sampling, sample preparation, rapid quantification of biomarkers and it will allow us to apply our technology to other space-based problems such as monitoring the spacecraft environment for chemical and biological contaminants.

We have also completed our initial studies on melatonin. In year three we will test melatonin in urine samples at this concentration level.

One of the specific aims of this project is to develop sampling and sample preparation techniques that enable the MALDI TOF mass spectrometer system to reliably detect, identify and quantify extremely low levels of chemical and biological substances in complex body fluids (urine, blood,

breath) with very low error rates. In year two, we reviewed several protocols for urine sampling and we have chosen a procedure to conduct measurements for year three.

Impact of these finding on technology objectives

We are developing and testing a small, efficient time-of-flight mass spectrometer coupled to a miniature gas chromatograph to rapidly identify important biomarkers and countermeasures for human space exploration. We are using the time-of-flight mass spectrometer to evaluate critical biomarkers and countermeasures that are indicators of bone loss, oxidative stress and the human sleep cycle associated for extended space travel.

Mass spectrometry is a technique for determining the masses of molecules and specific fragmentation products formed during vaporization and ionization. From detailed analysis of the mass distribution of the molecule and its fragments, molecular identification is accomplished. These molecular measurements can be carried out at the attomole (10^{-18} mole) level of material using specialized laboratory-based instruments. The combination of specific molecular identification and extreme sensitivity makes mass spectrometry one of the most powerful analytical laboratory tools yet developed for detection and identification of chemical and biological substances.

Proposed Research for Year Three

(1) Miniature Mass Spectrometry System

In year three, we will complete testing and evaluation of the new instrument system. We will use the results of this aspect of the project to make a recommendation as to the specific type of instrument that should be built in a follow-on program.

(2) Measurement of Oxidative Stress using TOF Mass Spectrometry

In year three, we will complete the study of oxidative stress biomarkers in urine samples.

(3) Zoledronate: a Countermeasure to Bone Loss in SCI Patients

In year three, we will analyze urine samples for zoledronate and related by-products.

(4) Assessment of Circadian Status Using the Miniature Mass Spectrometer

In year three we will analyze the urine samples from Dr. Kenneth P. Wright Jr. for the excretion of the melatonin metabolite, 6-sulphatoxymelatonin.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Yi-Xian Qin, Ph.D.
ORGANIZATION:	State University of New York – Stony Brook
PROJECT TITLE:	A Non-Invasive Scanning Confocal Ultrasonic Diagnostic System for Bone Quality

Project Executive Summary

The bone loss which parallels extended space missions represent serious threat to astronaut health, both during flight and on return to gravitational fields. Early diagnosis of osteopenia would enable prompt treatment and thus dramatically reduce the risk of fracture. The goal of this project is to develop a new technology for monitoring bone quality of humans during long-term space missions and on Earth. This will lead to a better understand of the progressive adaptation of bone loss in astronauts subject to microgravity and aging populations, and the ensuing musculoskeletal complications such as osteoporosis. Results of the joint Russian/US studies of the effect of microgravity on bone tissue demonstrated that bone loss proceeds at an average rate of 2 percent per month, ranging from no loss in the area of upper skeleton to as much as 14-20 percent loss in the skeleton of the lower body following a 14.5-month long mission. While these results are detected only when astronauts returned to Earth, the rate of bone loss *during* space mission is still unclear.

Currently funded by the NSBRI, we are able to develop a scanning confocal acoustic diagnostic (SCAD) system capable of generating acoustic images at the regions of interest (e.g., in the human calcaneus). This portable SCAD system is capable of generating non-invasive, high-resolution ultrasound (US) attenuation and velocity maps of bone, and thus determining the relationship between ultrasonic specific parameters and bone mineral density (BMD), and bone strength and bone's physical properties (i.e., stiffness and modulus). This system is relevant not only for ground-based determination of bone's physical properties, but can effectively be used in the space environment for determining even subtle changes in density and strength during extended flights. In this study, we plan to develop a 2-D ultrasound scanning system, and validate the structure and density information, detected by SCAD, using μ CT and mechanical testing methods in *ex vivo* animal models, as well as correlating to *in vivo* DEXA data derived from humans. The system will thus contribute to monitor degree and risk of bone loss in space and Earth, as well serve as a major step towards clinic usage as an early diagnostic of osteoporosis. There are proposed a series of four original specific aims (S.A.): (1) *Develop a scanning confocal acoustic diagnostic system for non-invasively mapping wave velocity and attenuation in bone;* (2) *Determine an interrelationship between ultrasound determined parameters, i.e., velocity and attenuation, and micro architectural parameters in a quantitative manner;* (3) *Develop a practical SCAD system for determining bone quality properties with quantified bone mass reduction;* and (4) *Map and monitor special directional and orthotropic strength of bone to predict BMD and structural modulus in vivo using the SCAD, and correlate these measurements to DEXA results.*

During this award year (2002-2003), the research team focused on continuing technology development of SCAD system (S.A.1 and 3) and validation between SCAD determined acoustic parameters and bone quality data (S.A.2). Human cadaver and in vivo subject testing were also initiated.

Technology Development

A system design, including hardware and software, of an experimental prototype was established which includes acoustic, electrical, control and mechanical components. As an important step towards a prototype for human testing, a 2-D SCAD system has been built including converging ultrasonic transducers, micro-controller controlled 2-D scanning stages, ultrasonic wave generator, low noise amplifier, and real-time analog/digital (A/D) transformer. A special designed controller is developed and synchronizes digital signals in acoustic wave, scan automation, and A/D transform. This micro-controller guided acoustic scanning technology greatly reduces the scan time, e.g., it requires only approximately *3-4 minutes for a 40x40 pixel array* in the region of interest to form images with 0.5~1 mm resolution. Ultrasonic attenuation and velocity images are obtained and calculated, e.g., gray scale or virtual color mapping.

SCAD as noninvasive modality for animal bone quality assessment: The ability of SCAD in non-invasively evaluating bone quality and quantity was tested in a large group of ex vivo bone samples. Trabecular samples were prepared as 1x1x1 cm cubes, which were harvested from sheep femoral distal condyle. These sheep were previously under a mechanical stimuli protocol for 1~2 years and identified distinct bone mineral density using dual-energy X-ray absorptionmetry (DEXA). All bone samples were mechanically tested by direct force-deformation in orthogonal directions, i.e., longitudinal, medial-lateral, and anterior-posterior, using a MTS universal test machine. The central plane of the samples was scanned with ultrasonic attenuation and velocity using SCAD system. The results of ultrasonic attenuation and velocity were correlated with mechanical moduli of the sample. While using a single transmitted ultrasound signal, there were weak correlations between measured BUA and micro-CT determined osteo-parameters, e.g., BMD ($R^2=0.28$), porosity ($R^2=0.28$), trabecular thickness ($R^2=0.04$) and trabecular space ($R^2=0.56$), as well as average modulus ($R^2=0.40$). These correlations were significantly improved using the SCAD system. Using SCAD system, μ CT and mechanical testing, new constitutive relations were derived using linear regression correlations in the results, which predict BMD and bone stiffness as the functions of acoustic parameters using combined BUA and UV as well as a series of rational constants. Strong correlations are observed between SCAD determined BUA and micro-CT determined parameters, i.e., BMD ($R^2=0.76$), porosity ($R^2=0.61$), structural mode index ($R^2=0.86$), and average modulus ($R^2=0.71$).

SCAD used for human calcaneus bone quality assessment: The feasibility of SCAD assessment for bone quality in the real body region, which include soft tissue, cortical bone and different surface morphology, is evaluated in cadaver calcaneus. 19 human calcaneus, harvested from cadavers with ages 66~97 have been imaged. BUA and ultrasound velocity determined from region of interest (ROI) have been performed. Bone samples were further tested for structural and strength parameters using μ CT and mechanical testing in the extracted cylindrical samples (10 mm in diameter and 20 mm in medial-lateral length) from ROI. Strong correlations were found between BUA and bone volume fraction (BV/TV) ($R^2 = 0.76$), and between UV and bone's modulus ($R^2 = 0.53$). The correlations are significantly improved ($R^2 > 0.64$) using combined parameters of BUA and UV in linear regression which ultrasound images determined parameters predict structural, e.g., structure morphological index (SMI) ($R^2 = 0.86$), and strength modulus ($R^2 = 0.64$).

These results suggest that high-resolution acoustic mapping is capable of predicting calcaneus bone quantity and quality non-invasively. Structural property parameters of trabeculi, e.g., BMD and BV/TV, is better represented by BUA, while ultrasonic wave velocity has a strong agreement with bone's strength property, e.g., modulus. Ultrasonic imaging has shown the great

potential to be used as *in vivo* diagnostic modality for assessing skeletal disorder, i.e., osteoporosis.

Pilot study for human bone quality assessment at large critical site, e.g., proximal femur, using SCAD and DEXA: To explore the potential of using SCAN to detect ultrasound bone image in the hip, human cadaver hip region is tested using an experimental SCAN system. This can evaluate the feasibility of ultrasonic assessment of bone quality *in situ*, which includes cortical bone and different surface morphology. The acoustic confocal region converged in the middle of the coronal plane of the hip with a focal zone approximately 0.5 mm in diameter in the focal region. Thus, a 2-D scan covers the central bone of the proximal hip. The confocal scan area covers an approximate $100 \times 100 \text{ mm}^2$ with a 0.5 mm increment. The signals transmitted through the bone are processed to calculate the slope of the frequency-dependent BUA (dB/MHz), the ultrasound attenuation (ATT, dB), and the ultrasound wave velocity (m/sec), and to generate BUA, ATT or UBV images. The data demonstrated that SCAD is capable to detect bone tissues in the critical skeletal sites, e.g., hip.

Impact

The results have demonstrated the feasibility and efficacy of SCAD for assessing bone's quality in bone (CRL 4 and 5). With proof of the concept using SCAD in bone quality assessment, we have filed two new technology disclosures through University's Technology Transfer Office. Five journal papers are either published or under review, and approximately 12 conference papers were published, which are directly derived from this work. We have been able to demonstrate that the bone quality is predictable via non-invasive scan ultrasound imaging in the ROI, and to demonstrate the strong correlation between SCAD determined data and μ CT identified BMD, structural index, and mechanical modulus. These data have provided a foundation for further development of the technology and the clinical application in this continuing research.

Research plan for the coming year

In the coming year, the research team will focus on (1) developing a practical SCAD system for determining bone quality properties with quantified bone mass reduction for clinical assessment, (2) assessing human calcaneus bone quality within a selected group, e.g., normal and osteoporosis subjects, to predict BMD and structural modulus *in vivo* using SCAD and DEXA, and (3) developing SCAD system for multiple sites testing, e.g., hip region.

A well-established SCAD system may provide a significant impact in diagnostic of osteoporosis and bone quality. Results may provide insight for addressing the risks of bone loss during prolonged space mission, age-related acceleration of osteoporosis, and monitoring healing of fracture.

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Veljko Radeka, Ph.D.
ORGANIZATION:	Brookhaven National Laboratory
PROJECT TITLE:	Heavy Ion Microbeam and Micron Resolution Detector
END DATE:	09/30/2004

Project Executive Summary

Project Aims:

Background and Significance. The use of microbeam provides a unique way to control the number of particles traversing individual cells and localizing the dose within the cell. High energy heavy charged particles transfer their energy to biological organisms through high density ionization and excitation along the particle track even by uniform irradiation. This characteristic microscopically non-uniform dose delivery is expected to induce complex DNA damage and mutagenesis, in contrast to relatively uniform dose delivery in gamma-rays or electron beam irradiation. To investigate the distinct biological effects of heavy ions, especially to determine the effects of occupational and environmental exposure of very low doses of heavy charged particles, for example, since virtually no cells receive more than one traversal of cosmic ray HZE particle in its lifetime in a spaceflight environment, one approach is to select cells with the desired exposures from a randomly irradiated population.

Using conventional track segment irradiation methods and sophisticated ion track detecting techniques, the position of the target cells and the ion tracks can be measured together. However, this conventional approach is not practical because all responses of many cells which do not contribute to the aim of the irradiation experiment must be measured. The alternative is to control each ion hit so that irradiation experiment is not a random Poisson process. A heavy ion microbeam can be used to selectively irradiate individual cells which can be analyzed afterward to determine what changes have occurred to that cell and to its un-irradiated neighboring cells.

Although the characteristic biological effects of heavy ions are supposed to be linked to the induction of high-LET-specific DNA lesions, other pathways of radiation effects are still interesting to consider, and there have been several reports that radiation effects of heavy ions may be transmitted from irradiated cells to neighboring unirradiated cells. A heavy ion microbeam can be used to look for pathways other than DNA damage, e.g., damage to the cell membrane or cytoplasm.

The localized dose delivery of a heavy ion microbeam can be applied to the inactivation of a microscopic region of target organisms, cell surgery technique. Heavy ion beams have been applied to radiation therapy development and radiation biology. For evaluation of radiation risk on mammalian cells, proton and helium ions with energies of a few and several MeV from Van de Graff accelerators are mostly used. However, heavy ion beams from those accelerators cannot be applied to the study because of their poor penetration.

Therefore, we want to design and test a high-energy microbeam apparatus and a micron-resolution solid state detector for space radiobiology studies. In addition, we will develop *in vitro* models relevant to radiation risk using a microbeam capable of delivering individual charged particles to individual cells *in situ*. The system will allow us to critically determine the response of human cells to the single-particle traversals typically encountered in space

environmental exposures, avoiding the confounding effect of the Poisson distribution of particle traversals inherent in conventional exposure systems. During long-term space flight mission, it is estimated that virtually no cell receives more than one *Fe* ion traversal in 3-year Mars mission scenario. Thus the use of the microbeam will aim to produce data for direct input into the analysis of human health risks during long-term space flights exposures involving exposure to low fluences of charged particles.

A single-ion microbeam facility comprises a number of elements arranged to deliver reliably counted numbers of ions to a chosen biological target. The elements are:

- a) a source of ions of the appropriate energy,
- b) a means of limiting the location of the ions to an area less than the area of the target,
- c) a means of locating and moving the biological targets to the beam position,
- d) a means of detecting each ion as it traverses the target, and
- e) a means of shutting off the beam after the arrival of the chosen number of ions.

A principal objective of this project is to develop and demonstrate a high resolution silicon detector, which will be able to determine the position of impact of energetic heavy ions in single cell radiation effects studies to within ~ 1 micron. An additional objective is a conceptual design of a heavy ion microbeam in the energy range up to 3 GeV. The beam will be collimated to $\sigma \sim 10$ -20 microns, to a region of one or very few cells. The microbeam will be implemented in a separate project at the BNL NASA Space Radiation Laboratory (NSRL), previously known as Booster Accelerator Facility (BAF).

These developments will advance significantly the state-of-the art of high energy heavy ion microbeams and of high resolution heavy ion detectors. For the cell studies employing these tools, the necessary infrastructure will include a micro positioning stage with a microscope and auxilliary detectors.

Results:

A new concept for a silicon detector with micron position resolution for heavy ions has been developed. First prototypes have been completed and initial testing performed. Basic design studies for the heavy ion microbeam in the energy range of several hundred MeV (and up to 3 GeV) at the BNL NSRL have been completed. Existing microbeams are all at much lower energies, less than 20 MeV.

Implications:

The micron resolution detector, together with the microbeam, will be able to localize the position of an ion impact within a particular region of the cell. This is essential for studies in space radiobiology.

Synergisms:

Radiation Effects Program, in particular, damage to central nervous system from radiation exposure. The objectives and specific aims of this project have been defined in consultation and with the agreement of the team working on radiation effects studies.

Milestones:

The milestones for the first two years of the project have been completed. In, particular, detector design has been developed, first prototypes completed, readout electronics with position interpolation has been developed, and basic microbeam design parameters have been achieved.

Research Plan for the Coming Year:

In the final year of the project, the processing of the second version of the detector prototype (which is expected to be the final version) will be conducted. Tests of the new detectors will be performed. Design study for the microbeam will be completed.

First half of the third year: Finish the processing of the second version of the prototype detectors.

Second half of the third year: Finish the tests (iron beam) of the new detectors. Produce the required number of detectors for the project.

In addition, a demonstration of the detector with cell samples (i.e., localization of incident single iron ions in particular regions of the cell) will be performed (consistent with the schedule of the heavy ion beam facility at NSRL).

RESEARCH AREA:	Technology Development
PRINCIPAL INVESTIGATOR:	Yu-Chong Tai, Ph.D.
ORGANIZATION:	California Institute of Technology
PROJECT TITLE:	Handheld Body-Fluid Analysis System for Astronaut Health Monitoring

Project Executive Summary

Body fluids such as urine, blood and saliva share the common attribute of being a suspension of biological elements. Normally, elements are monitored by their morphological appearance, surface markers or DNA. There is a tremendous amount of information in body fluids, and examining these fluids is a first important step of health monitoring. For example, blood analysis is a powerful technique to assess immunosuppression and disease, while low RBC count, hemoglobin and hematocrit are caused by anemia or internal bleeding. Body fluids must be prepared before analysis, and the preparation can be similar for each recognition method. Thus, a single platform technology may accommodate multiple body fluids and analysis methods.

Our long-term objective is to develop body-fluid analysis systems for space applications, emphasizing small form factor, lightweight and autonomous operation. For NASA, blood count is also important for astronaut health monitoring. Since Gemini and Apollo missions, it has been shown that astronauts can lose up to 20 percent of their red blood cell mass in just a few days. Unfortunately, these early experiments had to rely on inflight blood collection and post-flight analysis due to the lack of portable instrument. Even today, NASA still does not have an in-space blood count machine.

As a first step, the specific aim of this project is to develop an automated handheld blood-count machine. The system will use preloaded disposable cartridges so there will be no need for external buffer or reagent. The device will only need small blood samples (~50nL) that can be obtained through minimally invasive skin puncture and will use state-of-the-art integrated microfluidics technology. A monolithic chip will be developed to perform measurements of RBC count, MCV, hematocrit, WBC count and WBC differential. This platform technology could be extended to analyze other body fluids such as urine or to incorporate cell surface marker and DNA analysis.

**NSBRI RESEARCH PROGRAM
SPACE MEDICINE**

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RESEARCH AREA:	Space Medicine
PRINCIPAL INVESTIGATOR:	Harold K. Doerr, M.D.
ORGANIZATION:	Baylor College of Medicine
PROJECT TITLE:	Development and Testing of a Space-Adapted Human Patient Simulator

Project Executive Summary

The Medical Operational Support Team (MOST) was created by the National Space Biomedical Research Institute (NSBRI) and was tasked by the JSC Space Medicine and Life Sciences Directorate (SLSD) to incorporate medical simulation into 1) medical training for astronaut-crew medical officers (CMO) and flight control teams and 2) evaluations of procedures and resources required for medical care aboard the International Space Station (ISS).

The MOST has incorporated medical simulation into Space Medicine Operations by using the Human Patient Simulator® (HPS) manufactured by Medical Education Technologies, Inc. (METI, Sarasota, FL). The HPS is located in the Medical Simulation Laboratory (MSL) at Wyle Laboratories (Houston, TX). This MSL is a facility consisting of high-fidelity medical equipment and resources similar to those used aboard ISS. The MSL was developed and configured by the MOST to simulate the environment in which flight surgeons, BMEs (biomedical engineers/flight controllers) and CMOs would mitigate medical events on orbit.

In the past year, the MOST has introduced the MSL to multiple operational groups at JSC as well as the training approaches of medical simulation, in particular, Crew Resource Management (CRM). CRM is a communication system that was developed for the medical environment from communication techniques used in flight simulation, including critical thinking, critical communication and algorithm development. The MOST has begun to teach these concepts in concert with space medical training to both flight surgeons and BMEs. This effort is in line with the MOST's goal to develop a training curriculum for each of these flight controller disciplines in addition to a curriculum for Medical Flight Control Teams (flight surgeon, BME and CMO).

The efforts completed by the MOST in the past year have not been limited to curriculum development. The team has begun collaborating with an industrial leader in ventilation and a prominent military medical institution to conduct a study that would augment the standard of CPR care aboard ISS. In addition, the MOST has contributed to outreach events as demonstrated by its members being selected to be Plenary Speakers at the National Youth Leadership Forum (NYLF) Congress in July 2003. The MOST has also validated skill set training for surgical and non-surgical airway management as well as pneumothorax. The MOST has also developed a training regimen that allows NASA and Department of Defense (DOD) flight surgeons who are assigned for Russian Soyuz spacecraft recovery to administer anesthesia to a returning astronaut/cosmonaut "in the field" should it be necessary following the crew's return from an ISS mission. Recently, the MOST provided a Russian flight surgeon with refresher training in Advanced Cardiac Life Support (ACLS) in preparation for his ACLS exam. Taken together, the MOST has not only followed its intended objectives but has significantly expanded its role to the JSC Space Medicine Community.

The establishment of the MOST by the NSBRI has enabled SLSD to begin augmenting the standard of medical training for medical flight control teams and expand other areas of medical training. In the coming year, the MOST will continue this effort by not only validating its

training curriculums for the designated space flight disciplines but will also implement a Continuing Medical Education credit program for flight surgeons as per the guidelines of the American College of Graduate Medical Education (ACGME) and JSC Space Medical Operations. In addition, the MOST will implement video as an interactive medium to provide refresher training for medical flight control teams.

RESEARCH AREA:	Space Medicine
PRINCIPAL INVESTIGATOR:	Babs R. Soller, Ph.D.
ORGANIZATION:	University of Massachusetts Medical School
PROJECT TITLE:	Development and Testing of a Non-Invasive Sensor for Measurement of Muscle Metabolism During Exercise

Project Executive Summary

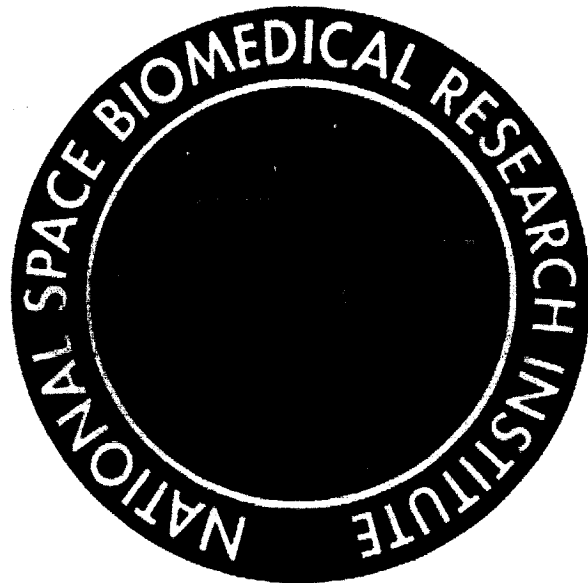
Physical exercise is the most important countermeasure currently used on the International Space Station (ISS) to mitigate muscle atrophy and bone loss, and to maintain cardiopulmonary function capacity. On ISS, assessment of health status is determined from a Periodic Fitness Evaluation (PFE) starting on flight day 14 and every 30 days thereafter, until the completion of the mission. This medical requirement is described in document, MR080L, Cardiovascular Physical Fitness Evaluation: Cycle Ergometry. Another medical requirement is certification for extravehicular activity (EVA). This test is conducted two weeks prior to a scheduled EVA. The test using arm cycle ergometry is described in document MR038L. In both tests, heart rate response in relation to ergometer workload is used to assess physical work capacity. However, the PFE calls for the measurement of oxygen uptake to assess aerobic capacity. Presently, the requirement for metabolism gas analysis is waived due to limitations of the analysis equipment. However, new technologies using near infrared spectroscopy (NIRS) to determine muscle oxygenation levels may provide a simple and straightforward measure of aerobic capacity. Thus, the goal of this project is to evaluate a new NIRS monitoring device for the assessment of crew health and performance capacities.

Near infrared spectroscopy (NIRS) can be used to simultaneously and continuously measure tissue oxygenation parameters, including muscle oxygen tension (PO_2) and muscle pH. These parameters can be measured noninvasively on specific muscles to continually assess muscle metabolism during exercise. Previous studies indicate that oxygen consumption, which is traditionally measured with a metabolic gas analyzer, can be calculated from near infrared spectra. This project will investigate the relationship between whole-body oxygen uptake and local tissue muscle oxygenation. The findings may allow reassessment of the need for metabolic gas analysis during the periodic fitness evaluation, and the use of heart rate during arm ergometry testing for certification of EVA. The device might also provide a quantitative assessment of pre-EVA hand-grip strength (MR081L, Physical Fitness Evaluation: Handgrip Dynamometry), where no metric currently exists.

This project will evaluate the accuracy and precision of NIRS-measured muscle PO_2 and pH for direct muscle measurement during exercise protocols used by NASA for astronaut fitness assessment pre-, in- and post-flight. The device will then be evaluated against standard measurements currently used (oxygen consumption, heart rate and workload) to develop models which relate the noninvasively measured NIRS-parameters to metrics currently used in NASA Medical Requirements for assessing physical fitness. The findings will enhance our understanding of the physiologic factors underlying leg and arm exercise. This information may lead to modification of ISS medical requirements which specify physical fitness procedures and equipment. These modifications may lead to reduced in-flight astronaut time spent on exercise countermeasures, fewer consumable resources, and greater accuracy in the assessment of physical fitness.

Appendix C

**NATIONAL
SPACE BIOMEDICAL
RESEARCH INSTITUTE**



***RESEARCH TEAM REPORTS
FY 2004***

**National Space Biomedical Research Institute
Research Team Reports
FY 2004**

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Bone Loss

Cardiovascular Alterations

Human Performance Factors, Sleep and Chronobiology

Immunology, Infection and Hematology

Muscle Alterations and Atrophy

Neurobehavioral and Psychosocial Factors

Neurovestibular Adaptation

Nutrition, Physical Fitness and Rehabilitation

Smart Medical Systems

Technology Development

National Space Biomedical Research Institute
Bone Loss Team
ANNUAL TEAM REPORT

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Project Title	Principal Investigator
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Project Title	Principal Investigator
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I. ABSTRACT

The musculoskeletal system is uniquely dependent on Earth's gravity. Although human adaptation to the microgravity environment has allowed astronauts to maintain overall function, the musculoskeletal system rapidly degrades once the force of gravity is removed. Several studies, American and Russian, have demonstrated that bone loss approximates 1-2% per month – although there are significant regional and individual variations. For example, there are reports that the range of trabecular and cortical bone loss in the tibia of MIR cosmonauts varied from 0% to 24% per month. More recently, studies from the first six expeditions to the International Space Station (ISS) have shown that rates of bone loss on the ISS are similar to those observed on MIR, despite the range of exercise countermeasures that are currently employed. Bone loss of this magnitude has also been observed in human bed rest studies and in individuals following spinal cord injury, and thus these two settings provide important analogs of bone loss during space flight. The loss of bone mass compromises bone strength, and diminished bone strength increases the risk of fracture, presenting a hazard to astronaut health and function and a threat to mission success. Countermeasures applied to date, including current in-flight exercise regimens, dietary and vitamin supplements, and pre-flight conditioning, have not prevented bone loss during flights of up to six months in duration.

The goal of the NSBRI Bone Loss Team is to develop an effective countermeasure to bone loss during long-duration space flight. This has been a year of considerable progress, which is highlighted in this report. Four new projects are now underway (Bateman, Cavanagh, Midura, and Schaffler) while two projects (Bloomfield and Smith) were awarded funding to continue for another 4 years and several projects are drawing to completion (Bolander, Isales, Karsenty, Rubin, Shapiro). This past year has produced over 13 publications, with several more *in press*, and high-profile representation at national conferences, including the Orthopaedic Research Society and the American Society of Bone and Mineral Research. Investigators have reported significant findings (see Section III. Team Accomplishments) including the efficacy of PTH as a preventative and rehabilitative therapy, the use of biomechanical stimuli to attenuate bone loss during periods of disuse, and positive effects of zoledronate and other bisphosphonates on bone mass and strength.

We are at a unique moment in the development of countermeasures for bone loss, where a number of factors dictate the future directions of the Bone Loss Team. These include the new NASA mission which provides a timeline for human exploration of the Moon and Mars, the considerable activity of the pharmaceutical industry directed towards therapeutic intervention for osteoporosis on Earth, the re-evaluation of present exercise regimens because they have not been effective in preventing bone loss to date, and the new NASA JSC thrust on bed rest studies. All of these developments suggest that the activities of the Bone Loss Team should be characterized not only by new understanding of the basic mechanisms underlying countermeasures to bone loss and their application in space and also by high countermeasure readiness level human subject studies both in space and in bed rest to examine new and established countermeasures that have been developed for osteoporosis on Earth. A goal for the next round of NRAs will be to attract such studies to the Bone Loss Team portfolio. In addition, we aim to strengthen existing links with other NSBRI teams, to initiate the process leading to publication of a monograph on Bone Loss in Space, to include members of a broader Space Medicine and Science community in the Team, and to support the flight and bed rest experiments of team members, in particular the in-flight anti-resorptive trial on which Dr. Shapiro is a co-investigator and the bed rest study of the effects of exercise being conducted by the Cavanagh laboratory.

II. INTRODUCTION

Four risks defined in the Critical Path Roadmap are within the purview of the NSBRI Bone Loss Team:

Accelerated Bone Loss and Fracture Risk Failure to recover bone lost during mission coupled with age-related bone loss can lead to osteoporotic fractures at a younger age. This is important for crew health in long-duration missions and for designing rehabilitation strategies. This risk has 12 enabling questions.

Impaired Fracture Healing Bone fractures incurred during and immediately after long duration space flight can be expected to require a prolonged period for healing, and the bone may be incompletely restored, owing to the changes in bone metabolism associated with space flight. This risk has 10 enabling questions.

Injury to Joints and Intervertebral Structures Fascia, tendon and ligament overuse or traumatic injury, joint dysfunction upon return to normal/partial gravity. Hypogravity changes to intervertebral discs may increase risk of rupture, with attendant back pain, and possible neurological complications. This risk has 4 enabling questions.

Renal Stone Formation Urine calcium concentration is increased due to increased bone resorption during hypogravity and to decreased urine volume during periods of dehydration. This risk has 3 enabling questions.

The current NSBRI Bone Loss team program (Figure 1) has projects which are addressing questions in three of the above 4 risk areas. The program includes both basic and applied research aimed at the development of countermeasures that may be tested during flight of animals or crew within the next 3-5 years. Project Countermeasure Readiness Levels (CRLs) range from Level 7 for testing countermeasures in a Zero Gravity Locomotion Simulator (ZLS) to Level 2 for basic research investigations of leptin and GIP function as potential hormonal targets for countermeasure development. The team has active interaction with the muscle and nutrition teams since personnel from each of these teams are co-investigators on each other's projects. Among other important linkages (Figure 1) are: a) contributions by flight surgeons to the projects of Bloomfield and Bateman; b) Support of the planned bisphosphonate flight experiment on which Shapiro is a co-investigator; c) Interaction with the exercise bed rest study in the Cavanagh laboratory; d) links between the Cavanagh and Midura projects and the new Center for Space Medicine at the Cleveland Clinic Foundation; e) Collaboration between NASA Glenn Research Center and the Cleveland Clinic Investigators. Drs. Isales and Zerwekh are collaborating to examine GIP levels in bed rest patients to determine if microgravity (bed rest) alters the dynamics of GIP secretion in response to nutrients.

Members of the Bone Loss Team are active participants in operational programs and planning at JSC. Additional efforts in this area have involved participation in a NASA/NSBRI committee planning the evaluation of clinical biochemical testing in astronaut crew before, during and after flight. Dr. Sue Bloomfield, Texas A & M University, was an active member of the Critical Path Roadmap development team which has revised and expanded issues of relevance to the Critical Path Roadmap program.

Dr. Smith and her colleagues are continuing their relationship with Leo Pharmaceuticals, the maker of EB1089, and have initiated a partnership with Organon Pharmaceuticals, the maker of

tibolone, an agent whose metabolites exhibit androgenic, estrogenic and progestinic activities, that is currently approved for use in humans.

Dr. Shapiro is participating as an investigator with Drs. Jeffrey Jones and Adrian LeBlanc in the preparation and presentation of a Supplemental Medical Objective (SMO) to the Countermeasure Evaluation and Validation Program, JSC. This project will implement testing bisphosphonate effects, alendronate and zoledronate, on the ISS. Dr. Shapiro also serves on a committee of the Institute of Medicine charged with reviewing the Critical Path Roadmap for NASA.

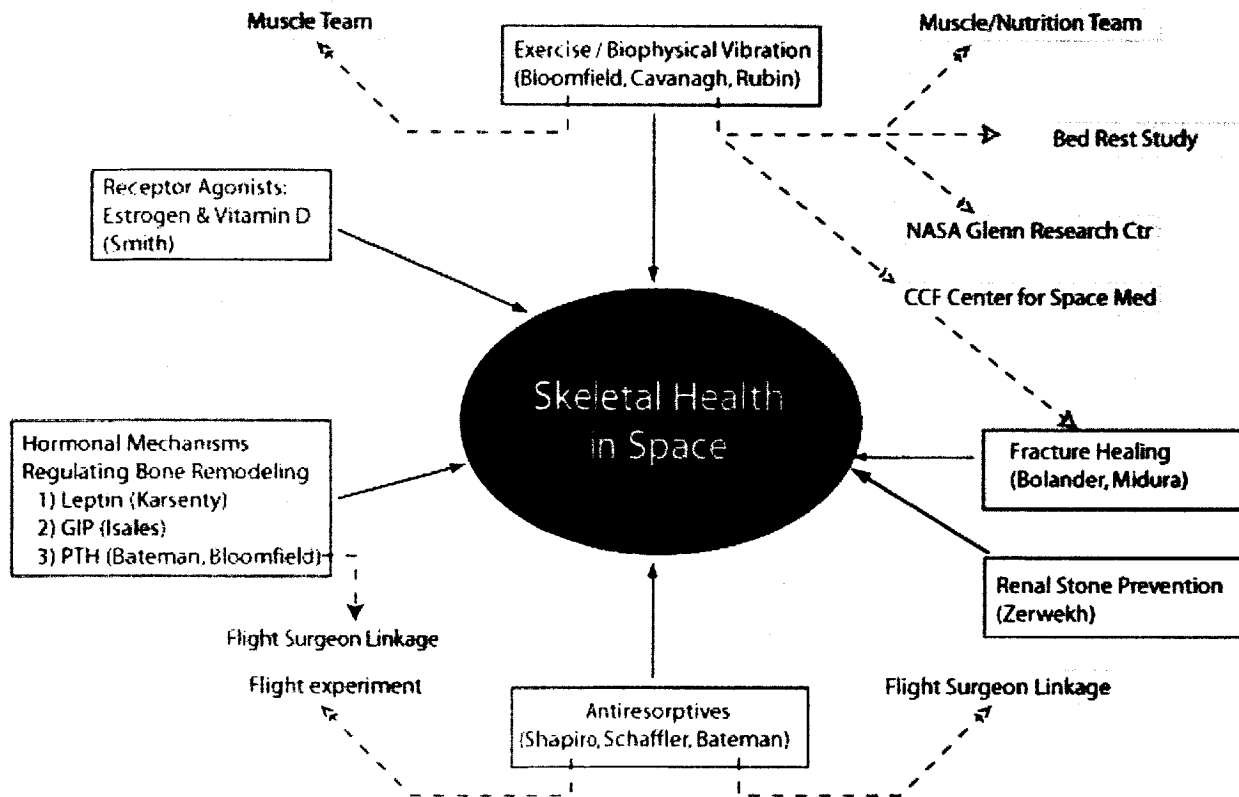


Figure 1. The NSBRI Bone Loss Team's contributions to Skeletal Health in Space and the linkages between team members and other related activities (see text for further details).

Given the centrality of humans in space exploration, the role of the Bone Loss Team is clearly well aligned with NASA's new Vision for Space Exploration. The Team has a clear and urgent primary mission to solve the problem of loss of bone mass in crew members during long-duration space flight and this task will be enabling to the entire program of exploration to the Moon and to Mars.

III. TEAM ACCOMPLISHMENTS

Overall accomplishments

As is apparent from the individual reports below, this has been a year of considerable progress for the Bone Loss Team. On average, at least one publication per month from team members based on NSBRI sponsored research has appeared in the refereed literature and a number of publications are in press. Several high profile presentations at national conferences have been given, including presentations at the Orthopaedic Research Society and the American Society of Bone and Mineral Research. Investigators have reported significant findings including the efficacy of PTH as a preventative and rehabilitative therapy, the use of biomechanical stimuli to attenuate bone loss during periods of disuse, and positive effects of zoledronate and other bisphosphonates on bone mass, geometry, and strength. The transition to new team leadership has been accomplished and four new projects are now underway (Bateman, Cavanagh, Midura, and Schaffler). Two projects (Bloomfield and Smith) were awarded funding to continue for another 4 years and several projects are drawing to completion (Bolander, Isales, Karsenty, Rubin, Shapiro).

Individual Accomplishments

In this section a brief review of the accomplishments of each PI who was a member of the team in the October 1, 2003 – September 30, 2004 period is presented (in alphabetical order by PI).

Bateman *Examination of Anti-Resorptive and Anabolic Treatments/Stimuli on Unloading Induced Osteoporosis.*

The purpose of this study is to examine the efficacy of zoledronate and osteoprotegerin (OPG) to prevent bone resorption. The study will further explore the effects of combining OPG with anabolic therapies. Three students have joined this project and have been trained in the animal procedures. One of these students is working on a similar project and will consult on this project for the first year to bring some more experience to the project. In addition to these students, a technician is being recruited to work on this project. The animals have been ordered for the first round of animal studies. After a review of the literature and receiving advice from representatives at Amgen, the team has decided to use an animal model of continuous administration of parathyroid hormone (PTH)-induced hypercalcemia rather than one of malignancy-induced hypercalcemia. This strategy will be used to help develop comparable, moderate doses of OPG and zoledronate.

Tangentially related to this project, the effect of intermittent and continuous administration of RANKL is being examined with the goal of determining the minimally physiologically active dose. It is hypothesized that very low doses may be anabolic (or at least increase turnover without large declines in bone mass). The continuous administration study has already begun and will continue for one month.

This project has already brought about successful interactions with Dr. Jeff Jones and Commander Dominic Gorie, both of Johnson Space Center. Dr. Jones and Commander Gorie toured the laboratory facility and were briefed on the project and goals. They both provided

important feedback and presented at the Bioengineering and Bioinformatics Summer Institute NIH/NSF undergraduate research program.

Publications:

None – new project as of April 1, 2004.

Bloomfield *Bone and Muscle Recovery after Simulated Microgravity*

These studies were designed to quantify the recovery of bone parameters and muscle functional properties in a muscle-bone pair following a standard 28 days of hindlimb unloading (HU) in the mature adult rat (6-mo-old males) and to explore strategies for stimulating recovery. The investigators had previously documented that muscle functional properties are fully recovered by 14 days of reambulation (normal cage activity) and published these findings on-line in the *European Journal of Applied Physiology*. A manuscript entitled “Mismatch of bone and muscle properties following long-term hindlimb unloading” is currently undergoing a third revision and will be resubmitted to *Calcified Tissue International* in the near future.

Because virtually all bone parameters (excepting periosteal bone formation rate of mid-shaft tibia) remain *depressed* below pre-HU values after 28 days of ambulatory recovery, the team extended these recovery studies to 84 days (three times the HU period) and are just now completing all the analyses of the “long-term” recovery animals. Total BMD of the proximal tibia, the most consistent marker of bone loss with HU, achieved about 50% recovery towards baseline (pre-HU) values. Results from histomorphometric measures and material properties testing of cancellous bone at the proximal tibia are being analyzed this month and will likely be published along with pQCT data in a separate manuscript to be submitted by December 2004.

The team’s most significant findings of the last year relate to the efficacy of a rehabilitative strategy targeted to promoting recovery of cancellous bone following HU, namely, that of intermittent parathyroid hormone (PTH). Daily injections of PTH (80 µg/kg BW/d) during 28 days of ambulatory recovery replaced all cancellous bone lost at the proximal tibia during unloading, with no deleterious effects noted on muscle functional properties. These findings were presented in an oral session at the October 2003 meetings of the American Society of Bone and Mineral Research. Recently completed material properties testing of cancellous bone from the proximal tibia confirms that the replaced bone has greater mechanical integrity as well.

These data have high relevance for rehabilitative strategies following prolonged space flight as well as for Earth-bound populations after prolonged bed rest or immobilization. Simple resumption of weight-bearing cannot be presumed to be adequate for recovery of bone lost, even as muscle function returns to normal. More aggressive, if short-term, anabolic therapies targeted to bone might be necessary to assure a parallel recovery of these two integrated tissues.

Publications:

Warren GL, Stallone JL, Allen MR, and Bloomfield SA (2004). Functional recovery of the plantarflexor muscle group after hindlimb unloading in the rat. *Eur. J Appl. Physiol.*

Narayanan R, Allen MR, Gaddy D, Bloomfield SA, Smith CL, Weigel NL (2004). Differential skeletal responses of hindlimb unloaded rats on a vitamin D-deficient diet to 1,25-dihydroxyvitamin D3 and its analog, seocalcitol (EB1089). *Bone*. 35(1):134-43.

Bolander *Effect of Microgravity on Fracture Healing/Ultrasound as a Possible Countermeasure to Bone Loss*

No report received.

Cavanagh *Foot Reaction Forces During Simulated ISS Exercise Countermeasures*

The goal of this joint project between the Cleveland Clinic Foundation and NASA Glenn Research Center (GRC) is to design a testbed that will allow the realistic simulation of on-orbit exercise. Design and fabrication of an enhanced Zero Gravity Locomotion Simulator (ZLS) has begun at GRC. The ZLS will be installed in the GRC facility to experimentally compare the effects on loads delivered to human subjects during exercise on floating, compliant, and grounded exercise devices. In preparation for installation, a ground reaction frame and air bearing slide have been designed and are currently in the final stages of fabrication. Extensive finite element modeling and review was completed before fabrication.

The subject load device (SLD) and harness are key components in the optimization of subject interface loading characteristics. Present evidence suggests that subject loads on-orbit during ISS exercise operations may be suboptimal, which could be a major reason for the lack of success of countermeasures using both iRED and TVIS. The project team has developed and is in the process of installing new feedback and control technology to achieve the constant load goal and will also explore the possibility of programmed variable loads to enhance ground reaction forces during countermeasure exercise. It should be noted that such controls allow the simulation of any level of gravitational acceleration, including greater than 1g and the lunar and Martian gravities of 0.16 and 0.37, respectively. The harness design will be addressed in the next year of the project.

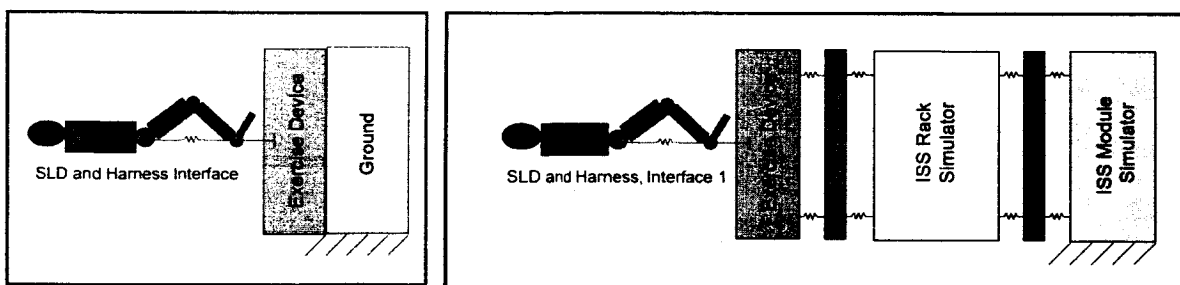


Figure 2. SLD and harness interface to device with grounded interface (as in the current ZLS, left) and the proposed enhancement with flight-like interfaces (right).

Publications:

None – new project as of February 1, 2004.

This study is designed to examine the effect of systemic Glucose-Dependent Insulinotropic Peptide (GIP) on bone homeostasis. Key findings during this period are divided into three areas: (1) Studies characterizing the GIP-overexpressing transgenic (GIP Tg+) mice; the investigators had previously demonstrated that GIP Tg+ mice have a 5% higher baseline bone mineral content than control mice at three months of age. Studies during this past year have demonstrated that biomarkers for bone formation (osteocalcin and alkaline phosphatase) are elevated and markers for bone breakdown (pyridinium (PYD) crosslinks) are decreased in GIP Tg+ mice consistent with an anabolic and antiresorptive GIP effect. In addition, in biomechanical testing, ultimate force in three-point bending is increased in GIP Tg+ mice consistent with GIP making these bones stronger; (2) Studies characterizing the GIPR KO mouse: the investigators had previously demonstrated that GIP receptor knockout mice (GIPR KO) have a lower bone mineral content than control mice. At one month of age, GIPR KO mice have a 39% lower BMC than control mice. When biomarkers were measured, GIPR KO mice had decreased markers of bone formation (alkaline phosphatase and osteocalcin) with markers of bone breakdown (PYD) which are the same compared to control mice. These findings are consistent with the GIP Tg+ mice data where GIP would appear to have both anabolic and antiresorptive properties; and (3) Studies characterizing the potential interactions between GIP and another anabolic agent, such as parathyroid hormone (PTH); to study the potential use of GIP in combination with other bone active agents, the team administered GIP, PTH, or the combination of GIP + PTH to C57/Bl6 mice for one month. The investigators found that the combination of GIP + PTH was more effective in increasing some markers of bone formation (alkaline phosphatase) and some biomechanical parameters (stiffness) compared to GIP or PTH alone. Thus, this approach holds promise that GIP could be used with other agents to maximize gains in bone mass.

Recently, the investigators have begun using an oral inhibitor of the enzyme that breaks down GIP (DPP-IV) to study if this compound has effects on bone turnover similar to those of GIP injection. If these effects are seen, then DPP-IV could be used alone or in combination with other countermeasures. This project has demonstrated that GIP is effective in preventing bone loss in ovariectomized mice. Hypogonadism is the most common cause of osteoporosis in humans; therefore, the investigators feel this project may influence the treatment of osteoporosis on Earth.

Publications:

- Chen JR, Chatterjee B, Meyer R, Yu JC, Borke JL, Isales CM, Kirby ML, Lo CW, Bollag RJ (2004). Tbx2 represses expression of Connexin43 in osteoblastic-like cells. *Calcif Tissue Int.* 74(6):561-73.
- Pi W, Yang Z, Wang J, Ruan L, Yu X, Ling J, Krantz S, Isales C, Conway SJ, Lin S, Tuan D (2004). The LTR enhancer of ERV-9 human endogenous retrovirus is active in oocytes and progenitor cells in transgenic zebrafish and humans. *Proc Natl Acad Sci U S A.* 101(3):805-10.
- Ding KH, Zhong Q, Xu J, Isales CM (2004). Glucose-dependent insulinotropic peptide: differential effects on hepatic artery vs. portal vein endothelial cells. *Am J Physiol Endocrinol Metab.* 286(5):E773-9.

Karsenty *Leptin as a Regulator of Bone Formation in Microgravity*

No report received.

Midura *Effects of Simulated Weightlessness on the Repair of Lower Limb Bone Fractures and on the Number of Bone-Derived Stem Cells*

The purpose of this study is to explore the effects of simulated weightlessness on fracture healing. In the first six months of funding, this team has implemented two independent trials to test the efficacy of parathyroid hormone (PTH) treatment in augmenting bone trauma healing in normal gravity. These trials involved bilateral 0.2-mm width osteotomies to the mid-diaphyseal region of the fibulae of 6-month-old female rats. Rats were permitted unrestricted cage activity (i.e., normal weight-bearing at normal gravity) and *ad libitum* food and water. PTH treatments consisted of a once-per-day subcutaneous injection of PTH₁₋₃₄ peptide at three dosages (20, 40, or 80 µg/kg body weight) and were compared to vehicle injection controls. The treatment regimen consisted of five injections per week over a four-week total test period starting one day after surgery. Bone healing in these live rats was assessed using *in vivo* micro-computed tomography (micro-CT). Imaging sessions were completed once per week over the entire four-week test period. The first trial is completed and is currently being analyzed quantitatively; the second trial of experiments is near completion and under qualitative review.

Using three criteria to assess bone healing (time of appearance, volume, and density of hard callus), the team's micro-CT results indicate that PTH treatment at 40 and 80 µg/kg body weight dosages exhibited a dose-dependent augmentation of bone healing as compared to either the vehicle or 20 µg/kg dosage groups. The results at the 80 µg/kg dosage were significantly better than those from the 40 µg/kg dosage group.

At the end of the test period, all tibiae and fibulae were recovered for (1) high-resolution micro-CT imaging *ex vivo*, (2) cantilever bending strength test of the healing fibulae, and (3) recovery of marrow-derived osteoprogenitors from the tibial diaphyses. During these recovery dissections it was observed that there was a PTH dose-dependent decrease in the amount of red marrow, with a concomitant increase in the amount of calcified marrow tissue, recovered from tibial diaphyses. Vehicle controls did not yield any noticeable calcified marrow tissue. Two notable observations that corresponded to the above-mentioned changes in the soft marrow tissues were a PTH dose-dependent increase in cortical thickness, and a dose-dependent decrease in the diameter of the intramedullary cavity of the tibial diaphysis.

Under normal gravity and weight-bearing conditions, and in a dose-dependent manner, PTH augmented fibular bone trauma healing, thickened the tibial cortex, and resulted in the recovery of calcified marrow tissue from the intramedullary cavity of the tibia. Thus, at this point in the study, intermittent PTH injections appear to be a potent anabolic therapy that augments the healing of severe bone trauma.

Publications:

None – new project as of April 1, 2004.

This project is exploring the possibility of harnessing bone's strong sensitivity to mechanical signals in order to provide a countermeasure for bone loss during long-duration space flight. There is increasing evidence that extremely low magnitude (<100 microstrain) mechanical signals can be strongly osteogenic if applied at a high frequency (15 to 60 Hz).

The principal objectives of this project have been to establish the efficacy of this unique, biomechanical countermeasure to inhibit bone loss in an animal model of disuse osteoporosis and correlate this regulatory influence to the expression patterns of several genes critical to bone formation and resorption. The PI believes that 10 min/d of these low-level signals (0.25 g), induced noninvasively using an oscillating platform, are able to retain bone mass despite 23 hours and 50 minutes of disuse, whereas 10 minutes of normal weight-bearing fails to do so. Longer-term animal studies (one year) have shown that low-level mechanical loading, inducing cortical strains on the order of 5 microstrain, can increase cancellous bone volume fraction, thicken trabeculae, increase trabecular number and enhance bone stiffness and strength. Considering these strain levels are far below (<1/1000th) those that may cause damage to the tissue, the investigators believe these signals hold great potential as a mechanical prophylaxis for osteoporosis.

Using the mouse as a model, it is also apparent that the genetic make-up of the animal is a strong determinant of their sensitivity to mechanical stimuli. Adult female 16-week-old C57BL/6J (low density), BALB/cByJ (medium density) and C3H/He (high density) mice were assigned to control, mechanically stimulated, and disuse groups ($n = 13$ each). Mice in the mechanically stimulated group were placed on a vibrating plate (45 Hz, 0.25 g) for 10 min/d. Disuse animals were subjected to unloading via tail suspension. Four animals per group were culled 4 days into the protocol for determining gene expression levels (semi-quantitative RT-PCR). The remaining animals were sacrificed after 21 days for the assessment of bone formation. Disuse failed to affect histomorphometric indices in C57BL/6J mice. In BALB/cByJ mice, mechanical stimulation increased bone formation rates by 34% ($p < 0.02$), but bone volume was unaffected. This increase in bone formation rate was primarily achieved by an increase in the ratio of double-labeled surface to single-labeled surface (+101%, $p < 0.001$). Disuse in the BALB/cByJ mice suppressed bone formation rates by 48% ($p < 0.01$), the ratio of double-labeled surface to single-labeled surface by 47% ($p < 0.01$), and mineral apposition rates by 45% ($p < 0.03$), resulting in trabecular bone volume that was 43% smaller ($p < 0.01$) than that of control BALB/cByJ mice. In contrast to the responsiveness of the skeleton of C57BL/6J and BALB/cByJ mice, no significant effects of mechanical stimulation or disuse were measured in tibial trabecular bone of C3H/HeJ mice. These tissue level results were essentially mirrored at the molecular level. The transcriptional levels of collagen type I, the most abundant protein in bone, were significantly reduced in tibiae of hindlimb-suspended BALB mice, but not in those of any other group. This finding further emphasizes the differential response of these mouse strains. The lack of upregulation of type I collagen mRNA after 4 days of mechanical stimulation may reflect its late occurrence in the cascade of events leading to new bone formation. Inducible nitric oxide synthase was significantly down-regulated by a similar percentage in mechanically stimulated mice of those strains that had responded to mechanical stimulation at the tissue level.

These data demonstrate that trabecular bone from C3H/He, BALB/c and C57BL/6 mice is differentially mechanosensitive and implies that, at the level of the human, some people may be more susceptible to developing osteoporosis, but that these individuals may be more responsive to biomechanically based interventions.

In summary, evidence in both animals and humans, at the molecular, histomorphometric, densitometric and structural levels, shows that short exposure to extremely low-magnitude, high-frequency loads has an anabolic effect. In essence, these studies lay the groundwork for a unique, nonpharmacogenic intervention for osteoporosis, based purely on the premise of “form follows function” in the skeleton and that these low-level signals can enhance both the quantity and quality of bone.

Publications:

- Judex, S., Garman, R., Squire, M., Donahue, L.R., Rubin, C.T. (2004) Genetically based influences on the site-specific regulation of trabecular and cortical bone morphology. *Journal of Bone and Mineral Research* 19(4), 600-606.
- Judex, S., Garman, R., Squire, M., Bhusa, B., Donahue, L.R., Rubin, C.T. (2004) Genetically linked site-specificity of disuse osteoporosis. *Journal of Bone and Mineral Research* 19(4), 607-613.
- Squire, M., Donahue, L.R., Rubin, C.T., Judex, S. (2004) Genetic variations that regulate bone morphology in the male skeleton do not influence its susceptibility to mechanical unloading. *Bone*. In press.
- Judex, S., Zhong, N., Squire, M., Garman, R., Donahue, L.R., Hadjiargyrou, M., Rubin, C.T. (2004) Mechanical modulation of molecular signals which regulate anabolic and catabolic activity in bone tissue. *Journal of Cellular Biochemistry*. In press.
- Zhong, N., Garman, R., Donahue, L.R., Rubin, C.T., Hadjiargyrou, M., Judex, S. (2004) Modulation in gene expression suppressed by disuse correlates with the disparate morphologic changes in the appendicular skeleton of two strains of mice. In review.

Schaffler *Bone Recovery Potential after Bisphosphonate and PTH Treatment of Disuse Osteoporosis*

This team's current research builds on the discoveries from the investigator's previous NSBRI research, conducted under the aegis of NSBRI BL00203 (*Resorption Suppression and Bone Health in Disuse*). These earlier studies reveal that anti-resorptive treatment with bisphosphonate reduced bone loss in disuse but did not completely inhibit it, consistent with results in acute spinal cord injury patients. These observations contrast with those for other osteoporoses in which bisphosphonate treatment effectively completely inhibits bone loss, suggesting that disuse is different from other osteoporoses in its sensitivity to antiresorptive treatment. After bisphosphonate treatment of long-term disuse osteoporosis, osteoclasts behave in a fundamentally different manner from normal and non-treated osteoporotic bone. These data suggest that disuse osteoporosis differs from other osteoporoses in its sensitivity to antiresorptive treatment.

The aims for the current phase of this research (BL00406) are to examine whether bone that remains after bisphosphonate treatment during long-term immobilization can recover its architecture and mechanical function after restoration of mechanical usage (remobilization). Specifically, the investigators will determine whether treatment conserves enough bone architecture to improve recovery and determine whether residual effects from bisphosphonates in the skeleton will adversely alter the biology of recovery once weight-bearing is restored. In the second series of studies, the investigators will assess whether the addition of anabolic 1-34 PTH

during remobilization will improve the bone recovery of bone mass *and* architecture in disuse bone.

In collaboration with a new post-doctoral fellow (Luis Cardoso Landa, Ph.D.), the investigators have initiated new studies on a novel, structurally based ultrasound procedure that significantly improves the prediction of mechanical properties by accounting for tissue anisotropy. Two abstracts from the team's first studies of disuse - bisphosphonate studies have just been accepted for presentation at the 51st Annual Meeting of the Orthopaedic Research Society in February 2005. Dr. Cardoso was selected to receive an NSBRI post-doctoral fellowship grant to expand these ultrasound studies. In the first phase of his work, he will use this novel, structurally-based ultrasound procedure to study bones from the team's first NSBRI experiment. The second series of studies will examine bones from the current resorption suppression/remobilization + PTH experiments, as they become available.

Publications:

Akkus O, Adar F, Schaffler MB (2004). Age-related changes in physicochemical properties of mineral crystals are related to impaired mechanical function of cortical bone. *Bone*. 34(3):443-53.

Li et al. (2004) Long-term disuse osteoporosis appears less sensitive to bisphosphonate treatment than other osteoporosis. *Journal of Bone and Mineral Research*. In press.

Shapiro *Defining and Preventing Bone Loss: A Microgravity Model*

Prolonged exposure of humans to microgravity causes significant lower-limb bone loss and similar patterns have been seen in tetra- or paraplegic spinal cord injury (SCI). In a double-blind, placebo-controlled, randomized study, these investigators evaluated the effects of intravenous 4 or 5 mg of zoledronic acid on SCI bone loss. Subjects included 12 males and 3 females (8 placebo, 7 zoledronate) evaluated within 10 weeks of injury and followed for 1 year. SCI lesions were located between C-5 and T-12 and were classified as ASIA A or ASIA B depending on motor/sensory deficit. DXA scans of the lumbar spine (LS) and hip were obtained at 0, 6 and 12 months using Hologic QDR 1000 and GE Lunar Prodigy densitometers standardized with a special phantom. Hip Structure Analysis was used to derive BMD, cross-sectional area (CSA), periosteal diameter, section modulus (Z), and estimates of endocortical diameter, mean cortical thickness and buckling ratio. Zoledronic acid maintained femoral neck CSA and had significant positive effects ($p < 0.05$) on BMD, CSA, periosteal diameter, Z, and buckling ratio in the intertrochanteric region. Overall, treatment effects were most evident at the shaft. Compared to placebo, treatment significantly reduced the decline in BMD, CSA, Z, and cortical thickness and attenuated the increase in endosteal diameter, and buckling ratio. Lumbar spine BMD rose 13.3% in controls and 5.2% in the treatment group: each group sat erect daily. Urinary n-telopeptide excretion was decreased following treatment. SCI, as a model for microgravity exposure, causes major losses in bone mass and structural strength at the proximal femur. Despite the small sample size, this study showed that zoledronic acid had significant positive effects on some structural parameters of the femoral neck and intertrochanteric region, and on all structural parameters in the femoral shaft, thus counteracting the negative effects of non-weight-bearing, and potentially that of microgravity, on BMD and bone strength.

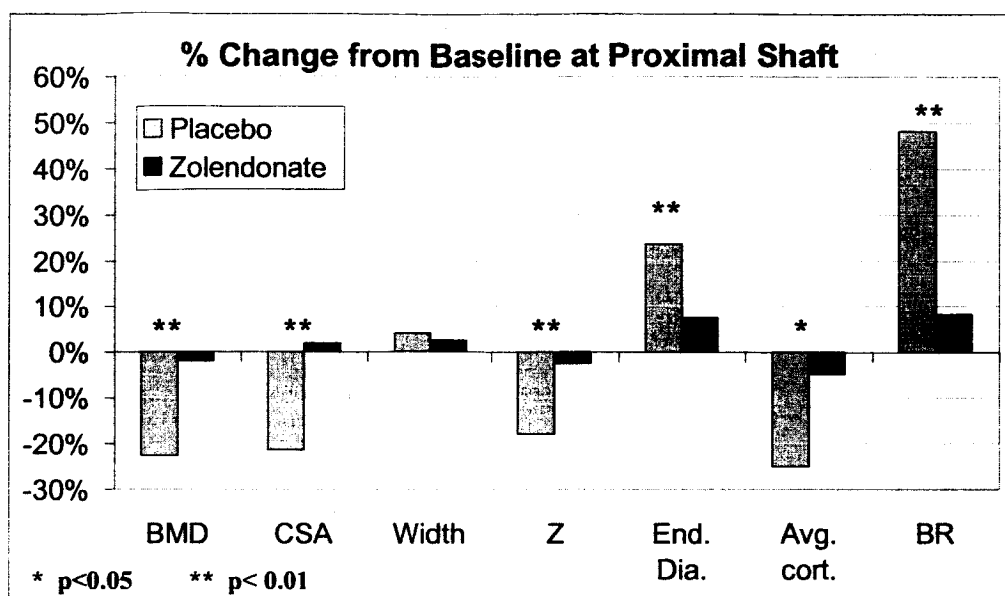


Figure 3. Changes in BMD and bone structural parameters in SCI patients with placebo and Zolendronate treatment.

Publications:

None.

Smith

Receptor Countermeasures to Disuse-Induced Osteoporosis

In the last year, this team completed the evaluation of two estrogen receptor (ER) and vitamin D receptor (VDR) agonists for their abilities, alone and in combination, to prevent bone loss in a hindlimb suspension model of disuse osteoporosis. In so doing, the investigators have obtained evidence that stimulation of the activity of each of these receptors can alleviate unloading-induced bone loss. Moreover, the investigators have found that the efficacy of the two ligands tested for the VDR, 1,25-dihydroxyvitamin D and EB1089, is gender dependent, with the former being significantly more effective in males than females, while EB1089 is much more effective in females than males. These effects are observed in bone, but also in the expression of calbindin-D9K, suggesting that promotion of intestinal calcium absorption may be a critical event for prevention of unloading-induced bone loss.

Space and Earth implications of this research include the demonstration that administration of ER and VDR based therapies can alleviate unloading-induced osteoporosis, and highlight the importance of evaluating countermeasures to space flight on males and females. Relative to Earth, the EB1089 data indicate that this agent has an anabolic effect on bone in females. These results suggest that this drug should be considered for treatment of postmenopausal osteoporosis, and that further study of the mechanisms of the gender-dependent response may provide insight in approaches to tailor bone-targeted therapies for men and women.

Publications:

- Lee CY et al. (2004) The combined regulation of estrogen and cyclic tension on fibroblast biosynthesis derived from anterior cruciate ligament. *Matrix Biology*, in press.
- Lee C-Y et al. (2004) Tensile forces attenuate estrogen-stimulated collagen synthesis in the anterior cruciate ligament. *Biochem Biophys Res Commun* 317:1221-1225.
- Narayanan R et al. (2004) Differential skeletal responses of hindlimb unloaded rats on a vitamin D-deficient diet to 1,25-dihydroxyvitamin D₃ and its analog, seocalcitol (EB1089). *Bone* 35:134-143.
- Narayanan R et al. (2004) The functional consequences of cross talk between the vitamin D receptor and ERK signaling pathways are cell specific. *J Biol Chem.*, in press.

Zerwekh *Prevention of Microgravity-Induced Stone Risk by KMgCitrate*

This team of investigators has performed an interim analysis of data collected during a bed rest study directed at assessing the efficacy of a “nutriceutical” countermeasure against renal stone disease. The analysis was made while maintaining the blind in 15 of the proposed 20 subjects to be studied, and the results were strongly supportive of the hypothesis proposed for specific aim 1 of the study, namely that alkali therapy as potassium magnesium citrate would reduce the relative saturation of urine with respect to calcium oxalate and undissociated uric acid as compared to placebo-treated subjects. Although the investigators did not see any significant difference in bone turnover markers between the placebo and KMgCitrate-treated subjects, they are hoping that with the completion of all 20 subjects, sufficient power will be attained to demonstrate a meaningful reduction in bone turnover. The interim analysis was also made possible through the support and help of the nurses and staff of the General Clinical Research Center who have provided the bedrest facilities, dietary, nursing, and laboratory support and have helped leverage the resources of the current grant. Dr. Zerwekh presented these interim results as a plenary talk at the Tenth International Symposium on Urolithiasis, which was held in May 2004 in Hong Kong. The talk was entitled “Nutrition and Renal Stone Disease; Lessons Learned from Space.”

Encouraged by findings on the interim analysis, the investigators have recently submitted an application to NASA in response to announcement number NRA-04-OBPR-01 in which the team proposes to conduct placebo-controlled evaluation of KMgCitrate in astronauts under long-term space station flight conditions. This application represents the next logical step in the evaluation and delivery of a potentially effective and safe countermeasure against renal stone disease. If this countermeasure is found to be effective in reducing the risk of renal stone formation under the rigors of microgravity, it may have a far greater benefit in reducing the risk and morbidity of renal stone disease on Earth.

Publications:

- Zerwekh JE, Odvina CV, Pak CYC. Prevention of microgravity-induced renal stone risk by potassium-magnesium-citrate(KmgCit) In: *Kidney Stones: Inside and Out*. Proceedings of the 10th International Symposium on Urolithiasis. Edited by DI Gohel and DWT Au. The Reprographic Unit, Kowloon, Hong Kong; 337-339, 2004.

IV. TEAM PLANS

Individual Plans

Bateman In the next 12 months, the team will determine physiologically equivalent doses of OPG and zoledronate that are minimal, but efficacious. They will also examine the short- and long-term effects of these two agents, at physiologically equivalent doses, on bone turnover (primarily bone formation). By the end of the fiscal year, a hindlimb-suspension study will be performed examining two of these doses for their ability to prevent disuse osteoporosis.

Bloomfield To complete Specific Aim 1, these investigators are constructing a replica of the rat flywheel device with some mechanical improvements to test the efficacy of voluntary resistance training with the more precisely controlled stimulated muscle-contraction paradigm during a 28-day period of hindlimb unloading in adult male rats. Preliminary strain gage studies will be performed to achieve comparable loading magnitudes with the two training paradigms. Primary outcomes in these studies will be pQCT measures of bone density and cross-sectional geometry, histomorphometric measures of bone formation rate, and mechanical testing of bone from the proximal tibia, a site that is particularly responsive to unloading. That training mode achieving the best mitigation of bone loss during 28 days of hindlimb unloading will then be utilized for subsequent years of this project.

Cavanagh In the upcoming year, this team will complete installation of the ZLS at NASA GRC and design a new harness. During this phase of the study, the team will explore design changes in the present ISS treadmill harness to improve subject comfort at higher SLD loads. Past experience in harness design has led the investigators to believe that a load-distribution device must be inserted between the SLD and the body. The team further plans to develop and benchmark rigid-body and nonrigid-body mathematical simulations of countermeasure interfaces. The goal of the mathematical simulations is to explore the sensitivity of the applied force at the foot of an exercising crew member to variations in interface parameters. This analysis will provide a perspective on which to base the experimental work to be performed. The leverage of NSBRI resources against life science activities at NASA GRC and the Cleveland Clinic's Center for Space Medicine should lead to added value for the NSBRI grant.

Isales During the next year, these investigators will concentrate on the use of the DPP-IV inhibitor and its effects on bone turnover. In particular, they plan to use it in tail-suspended mice.

Midura This group will fully analyze the recovered fibulae and tibiae from these normal-gravity groups. This analysis will include: a) high-resolution micro-CT imaging; b) *ex vivo* cantilever bending strength testing of healing fibulae; and c) osteoprogenitor colony-forming unit counts from tibiae. They will also initiate a similar research design for simulated microgravity groups involving tail suspension for 4 weeks followed by bilateral fibular osteotomies, $\pm$ PTH therapy.

Rubin A revised renewal application is currently under review by NSBRI. The goals of that proposal are to examine how low-level mechanical signals can augment the pharmacologic influence of antiresorptive (bisphosphonates) and anabolic (PTH) interventions.

Schaffler The investigator will complete immobilizations and begin remobilization studies for risedronate-treated animals. He will also begin PTH dose optimization studies and will continue ultrasound-based analyses for bone samples for previous studies.

Shapiro This investigator will continue to develop plans for the SMO flight experiment involving the administration of anti-resorptive therapy to humans. He has also submitted a proposal to NIH to study mechanisms and treatment of bone loss in individuals with spinal cord injuries more than one year after injury (correlation to bone loss on a 3-year flight).

Smith In the coming year, the goal of this project will be to formally test whether there are sex-dependent differences in the response to vitamin D receptor agonists, and in so doing determine whether countermeasures against disuse-induced osteoporosis should be developed and/or evaluated relative to gender. In addition, the group will begin the characterization of the effects of unloading on endocrine/calcium parameters, anterior cruciate ligament, bone marrow osteoprogenitors and bone, with the latter including analyses of global alterations in gene expression.

Zerwekh The funding for this grant officially ended September 30, 2004. However, Dr. Zerwekh has asked for and been given a 1-year no cost extension on the grant to permit Dr. Zerwekh to finish evaluation of the last two subjects needed to complete the projected number of 20. Approximately half-way into the study, Dr. Zerwekh was asked by the GCRC program director to limit his research bed use to one subject per admission versus the two that he had been doing. Because of this, he was not able to complete all 20 subjects by the end of the funding period. Thus, his plans will call for finishing subjects 19 and 20 by the spring of next year and then beginning data analysis and paper preparation. This stage will also include analysis of the serum GIP levels being performed in collaboration with Dr. Carlos Isales, a previously funded NSBRI investigator.

Overall Team Plan

We are at a unique moment in the development of countermeasures, where a number of factors dictate the future directions of the Bone Loss Team. 1) The schedule for continued human exploration of the Moon and for a mission to Mars have been defined, and the urgency of solving the bone loss problem is now sharply drawn as a result of NASA's new exploration mission. 2) There is a great deal of activity in the pharmaceutical industry directed towards therapeutic intervention for osteoporosis on Earth, and, in many respects, this activity is in advance of previous work in Space Medicine. 3) Exercise regimens are currently being re-evaluated because they have not been effective in preventing bone loss to date. 4) NASA has developed a centralized bed rest facility, which will offer opportunities for bone loss researchers to test their countermeasures with adequate statistical power.

All of these developments suggest that the activities of the Bone Loss Team should be characterized by a sound understanding of the basic mechanisms underlying countermeasures to bone loss and their application in space and by high CRL human subject studies, both in space and in bed rest, that examine new and established countermeasures that have been developed against osteoporosis on Earth. Traditionally, the Bone Loss Team has been strong in basic studies, particularly *in vivo* animal models. Such studies are well represented in the present portfolio of grants, and we expect them to deliver import and relevant results. Three human studies (Cavanagh, Shapiro, and Zerwekh) and one Supplementary Medical Objective (SMO) are

currently in the Bone Loss Team portfolio. At the next NRA, we hope to add projects at high CRLs that will test the efficacy of present therapies for osteoporosis in a space flight analog, most likely bed rest. Consideration will also have to be given to the addition of projects examining soft tissue injury – an area that is currently absent from the Team’s portfolio.

We will continue to broaden the input to the Bone Loss Team. This process was started by the inclusion of two nationally renowned bone experts, Dr. Christopher Jacobs from Stanford University and Dr. Charles Turner from Indiana University, as consultants at the Team meeting held in October 2004 in Seattle. Dr. Cavanagh also gave Grand Rounds to the JSC Flight Surgeons in September 2004, an opportunity that established good initial linkage with this important constituency. A number of astronauts have expressed interest in various team experiments, and their input will be nurtured during the coming year.

Our consultants have suggested that we attempt to establish a Program Project-like environment in which there is more synergy between projects. They have also recommended that we accelerate the bed rest trials of current pharmacological therapies. Both of these recommendations will be explored in the coming year.

Finally, the publication of an edited book on bone loss during space flight with contributions from Team members and others is being actively pursued.

The Bone Loss Team is cognizant of the fact that we have a clear and urgent primary mission to solve the problem of loss of bone mass in crew members during long-duration space flight. Solution of this problem by the provision of deliverables in a time frame compatible with the new exploration mission requires a narrowing of focus and a pragmatic approach that puts strong emphasis on projects with high CRLs, while nurturing a smaller number of studies on key future issues. Once significant progress towards solution of the primary problem has been achieved, more resources can be directed to other important long-range topics, such as fracture repair and skeletogenesis in microgravity.

CARDIOVASCULAR ALTERATIONS TEAM

National Space Biomedical Research Institute

Annual Program Report

October 1, 2003 – September 30, 2004

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Listing of Team Projects

Effects of Simulated Microgravity on Cardiovascular Stability (1)

Effects of Space Flight on Cardiovascular Stability (2)

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I. ABSTRACT

The Cardiovascular Alterations team is dedicated to the study of the adverse effects of space flight on the cardiovascular system and the development of effective countermeasures to these adverse effects. In conjunction with this effort the team develops new cardiovascular technologies which have spin-off benefits for use here on earth.

The adverse effects of space flight on the cardiovascular system include:

- 1) Impaired Cardiovascular Response to Orthostatic Stress
- 2) Occurrence of Serious Cardiac Dysrhythmias.
- 3) Diminished Cardiac Function
- 4) Manifestation of Previously Asymptomatic Cardiovascular Disease
- 5) Impaired Cardiovascular Response to Exercise Stress

The team currently involves eight closely integrated projects. The projects include two animal studies, two ground based human studies, two flight studies, and one computer simulation project. The projects interact with each other as well as with investigators in a number of other NSBRI teams. The team is focusing on the first three adverse effects with lower priority being assigned to the last two adverse effects.

The team has been successful in moving projects along from studies of basic mechanisms to the proposing and testing of countermeasures. The team is now actively evaluating ten specific countermeasures. Many of these countermeasures are at Countermeasure Readiness Level (CRL) 7. One the countermeasures to post flight orthostatic intolerance, the alpha-sympathetic agonist midodrine, was originally studied by the team in animal and computer simulations and then in a randomized double blind bed rest study. This countermeasure is now in flight testing (CRL 8) with a demonstration that it eliminated post flight orthostatic intolerance in one individual who was previously susceptible.

In addition, the team has developed six new cardiovascular technologies. One of these technologies, microvolt T-wave alternans (MTWA) testing, has been successfully commercialized and is in widespread clinical use for the identification of patients at risk for sudden cardiac death from ventricular arrhythmias. This past year Medtronic, Inc. launched a multi-million dollar 1500 patient trial using MTWA to identify a broader group of patients who may benefit from this life saving technology.

This year the team developed a new technology for the continuous monitoring of cardiac output. This technology will enhance cardiovascular space research and also provide an important tool for space and earth medicine for monitoring of patients who may be hemodynamically unstable. A patent has been applied for and commercialization discussions have begun.

This coming year the team plans to continue its efforts to bring the best science and technology to bear on mitigating the adverse effects of space flight on the cardiovascular system and improving cardiovascular health in space and on earth.

II. INTRODUCTION

During space flight the cardiovascular system undergoes adaptive changes in structure and function in response to weightlessness and other factors, such as sleep disruption, confinement and additional environmental alterations. Space flight is associated with a movement of fluid from the lower extremity to the thorax and head, a modest decrease in intravascular volume, and a modest decrease in arterial pressure. In addition, there are alterations in the lymphatic, neural and hormonal control systems. While these adaptations appear to be associated with generally adequate cardiovascular performance during conditions of short-duration space flight, they are not appropriate upon reentry into a gravitational environment. Furthermore, the extent of cardiovascular adaptation appears to increase with duration of space flight, and the magnitude and implications of these adaptations for long-duration space flight (that is, months to years) remain largely unknown.

Specific adverse effects of space flight on the cardiovascular system include:

1) **Impaired Cardiovascular Response to Orthostatic Stress** Upon reentry into the Earth's gravitational field, astronauts experience orthostatic intolerance, which limits their ability to function during reentry. In many cases, the orthostatic intolerance is sufficiently severe that astronauts cannot stand erect for some time after landing and thus may interfere with the ability of astronauts to egress from the spacecraft under emergency conditions. Upon reentry into a gravitational field blood pools in the dependent veins and arteries which leads to reduction in preload to the heart resulting in a decrease in stroke volume, cardiac output and arterial blood pressure. Factors involved in the development of orthostatic intolerance may include structural and functional adaptations of the heart and arterial and venous blood vessels and lymphatics, alterations in volume control mechanisms, alterations leading to an inadequate or defective neural and hormonal regulatory response, alterations in local vascular reactivity, and mechanisms controlling regional distribution of blood volumes and flows. Factors such as age, gender, genotype, as well as occupational, physical training and dietary history may affect individual susceptibility to the development of post-flight orthostatic intolerance. Currently-used countermeasures, such as oral administration of salt and water prior to reentry and application of anti-gravity suits, do not adequately prevent orthostatic intolerance, especially following long-duration space flight.

2) **Occurrence of Serious Cardiac Dysrhythmias** A number of anecdotal reports suggest that long-duration space flight might lead to an increased incidence of potentially serious heart rhythm disturbances. If space flight does in fact significantly decrease cardiac electrical stability, the effects could be catastrophic, potentially leading to sudden cardiac death. It will be important to determine the mechanisms underlying this phenomenon in order to develop appropriate countermeasures. Potential mechanisms that might lead to reduction in the stability of the electrical substrate include electrolyte changes, changes in the neural and hormonal milieu, and alterations of cardiac myocytes, myocyte connectivity and extracellular matrix resulting from space flight. These alterations may in turn lead to changes in cardiac conduction and repolarization processes which predispose to sustained rhythm disturbances.

3) **Diminished Cardiac Function** Long-term space flight may lead to a measurable reduction in cardiac mass, probably associated with cardiac remodeling. It is not known whether these cardiac alterations are reversible and whether they pose a long-term health risk to astronauts. Factors that may be involved in alterations in cardiac function include changes in myocyte number, size, and geometry; changes in myocardial matrix and microvasculature; alterations in myocyte and organ-level mechanical performance; changes in cardiac gene programming; stimuli and signals that lead to loss of cardiac mass and remodeling; factors affecting reversibility and recovery from these alterations.

4) **Manifestation of Previously Asymptomatic Cardiovascular Disease** Long-duration space flight may exacerbate previously undetected cardiovascular disease, such as coronary artery disease. Little is known about what conditions of space flight may tend to make pre-existing disease symptomatic or accelerate the progression of the underlying disease. Also, we do not know what procedures should be applied to screen astronauts for the presence of asymptomatic cardiovascular disease prior to long term missions

5) **Impaired Cardiovascular Response to Exercise Stress** Long-term space flight may impair cardiovascular response to exercise. Current inflight exercise programs appear adequate to maintain aerobic exercise capacity.

The Objectives of the NSBRI Cardiovascular Alterations Team are to:

- Characterize and quantify the adverse effects of space flight on cardiovascular structure and function
- Determine the mechanisms of these adverse effects
- Develop effective countermeasures to these adverse effects
- Develop new cardiovascular technologies for use in countermeasure development and for spin-off applications on earth

The Critical Issues faced by the Cardiovascular Team in addressing the Critical Risks include:

- Development of Suitable Experimental Models
- Development of Suitable Experimental Approaches
- Development of Mathematical and Computer Models
- Development of New Cardiovascular Technologies
- Addressing the Multiple Conditions Imposed by Space Flight
- Countermeasure Development Issues
- Determinants of Individual Susceptibility to the Adverse Cardiovascular Effects of Space Flight
- Development of Spin-off Technologies to Benefit Clinical Medicine on Earth
- Development of a Space Flight Database

Team Structure and Design

The overarching intentions of the Cardiovascular Alterations Team are to:

- Characterize and quantify the adverse effects of space flight on cardiovascular structure and function

- Determine the mechanisms of these adverse effects
- Develop effective countermeasures to these adverse effects
- Develop new cardiovascular technologies for use in countermeasure development and for spin-off applications on earth

The program's overall strategy is dictated by the relevant risks: The following risks are deemed to be high priority and are the focus of the team's efforts:

- Impaired Cardiovascular Response to Orthostatic Stress
- Occurrence of Serious Cardiac Dysrhythmias
- Diminished Cardiac Function

The remaining two risks are deemed to be of lower priority:

- Manifestation of Previously Asymptomatic Cardiovascular Disease
- Impaired Cardiovascular Response to Exercise Stress

While some of the projects do address some aspects of these two risks, no one project principally addresses these risks.

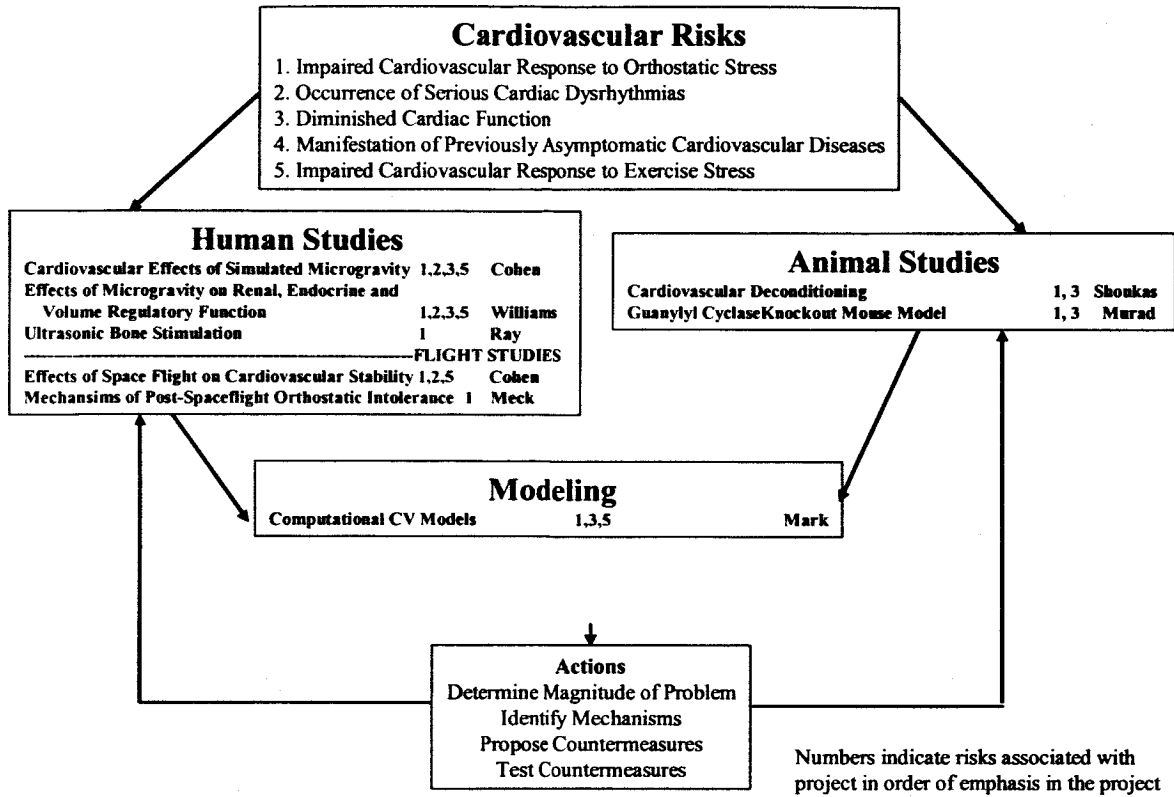
Additional non risk-based goals include those associated with the development of new cardiovascular technologies for use in space and for earth based applications.

The current program is summarized in the Figure below. The projects are organized in three areas: animal studies, human studies (ground based and flight), and computer modeling. We are pleased that our team has two flight studies which have been approved and funded. The computer modeling is used to analyze data from the experimental studies and to refine hypotheses to be tested experimentally.

The size of team has been reduced from 16 projects in 2003 down to 8 projects in 2004. The previous large size of the team was due in part to a number of projects that were reassigned to the team as a result of the elimination of the Integrated Systems Physiology team. The current projects within the team are well integrated with one another and we also have close interactions with a number of the other teams including Smart Medical Systems, Neurovestibular, Space Medicine, and Human Performance. We are also currently planning research activities related to the effects of radiation on the cardiovascular system.

Our team efforts have been organized to support the Vision for Space Exploration. Our research is geared to addressing critical risks and developing countermeasures for low earth orbit missions, as well as missions to the Moon and Mars and beyond.

FIGURE: CARDIOVASCULAR ALTERATIONS TEAM



III. TEAM ACCOMPLISHMENTS

Overview

Below we provide a summary of the accomplishments of the Cardiovascular Alterations Team, followed by reports of progress for each individual project.

Countermeasure Development

Our team is highly oriented towards countermeasure development. Below are a list of the countermeasures being developed. Many have progressed to Countermeasure Readiness Level (CRL) 7, and one (midodrine) has progressed to CRL level 8.

Orthostatic Intolerance Countermeasures (CRL Level)

- Midodrine (7, 8)
- Single Bout Maximal Exercise (7)
- Salt and Water Loading (7)
- Ultrasonic Bone Stimulator (6)
- NO/cGMP Inhibitors (5)
- Cardiovascular System Identification Screening (7)

Cardiac Dysrhythmias Countermeasures (CRL Level)

- Potassium and Magnesium Supplementation (7)
- Eplerenone (7)
- Metoprolol (7)
- Microvolt T-Wave Alternans Screening (7)

The Cardiovascular Alterations Team conducted the first ground based evaluation of the alpha-sympathetic agonist midodrine as a countermeasure to microgravity induced orthostatic intolerance. The team first evaluated this countermeasure in animal studies and computer simulations. Then it conducted a randomized double-blind bed rest study which demonstrated that midodrine dramatically reduced post bedrest orthostatic intolerance. The midodrine countermeasure has already been tested in flight under a Supplemental Medical Objective and was found to be extremely effective in eliminating post flight orthostatic intolerance in one subject who was very orthostatically intolerant after previous flights. The midodrine countermeasure is particularly attractive in that it involves taking only a single pill at the very end of flight, and thus does not interfere with appropriate cardiovascular adaptation to weightlessness during the flight itself.

Earth Benefit Deliverables

The Cardiovascular Alterations Team is also highly focused on developing spin-off technologies for medical benefits here on earth. Below is a list of these technologies which are at various stages of development.

- Microvolt T-Wave Alternans for Prevention of Sudden Cardiac Death
 - Commercialized and in Widespread Clinical Use
- Strategies for Treatment of Orthostatic Intolerance on Earth

- Cardiovascular System Identification for Assessment of Closed-Loop Cardiovascular Regulation in a Variety of Disease States
- Venous Pump G-Suit for Treatment of Low Cardiac Output
- Cardiovascular Computer Models and Hemodynamic Databases for Research and Clinical Use
- Continuous Cardiac Output Monitoring

The Cardiovascular Alterations Team developed the technique of Microvolt T-Wave Alternans testing in order to assess the effect of microgravity on the susceptibility of the heart to life threatening heart rhythm disturbances. This technique has been cleared by the FDA, is reimbursed for by Medicare, successfully commercialized and in widespread clinical use for the reduction of sudden cardiac death here on earth. It has been evaluated in numerous clinical trials performed around the world, including a multimillion dollar 1500 patient MASTER study currently underway which is sponsored by Medtronic, Inc. The purpose of this trial is to expand the population of patients who may benefit from this technology.

A second technology for continuous monitoring of cardiac output has been recently developed. This technology will enhance cardiovascular space research and also provide an important tool for space and earth medicine for monitoring of patients who may be hemodynamically unstable. A patent has been applied for and commercialization discussions have begun.

Summary of Progress on Strategy

- Good overall progress has been made on the strategic plan, with solid scientific advances made in each of the three targeted cardiovascular risk areas as well as in the cross-cutting areas of modeling and new technology development.
- The team's progress is extensively documented in the premier scientific and engineering journals and in the patent literature. The record of these publications may be found in the individual project reports.

Tactical Plan Summary

- The team collaborates effectively and has good interactions with other NSBRI teams and outside investigators. In particular we interact with Vestibular, Smart Medical, and Human Performance teams and are engaged in discussions regarding interaction with NASA's effort in radiation.
- The two flight studies have been moving along well in terms of working through, together with the JSC staff the detailed preparation process for flight. Experiment documents have been prepared, multiple approvals have been obtained, the experiments have been presented to flight crews, and dry runs of the data collection protocols have been conducted.
- This past year the Richard Cohen taught in a summer life sciences course for astronauts held at Woods Hole. Through the flight studies and this type of educational activity we plan to continue to increase our contact with the astronaut corps.

Accomplishments by Project

Effects of Simulated Microgravity on Cardiovascular Stability: Richard Cohen, P.I.

Specific Aims of the previous project:

1. To investigate quantitatively and non-invasively alterations in cardiovascular regulation and function during and after simulated microgravity exposure using the technique of Cardiovascular System Identification (CSI). The general hypothesis being tested in this specific aim is that CSI measures of closed-loop cardiovascular regulation are altered after bed rest.
2. To apply Microvolt T-Wave Alternans (MTWA) analysis to investigate the impact of exposure to simulated microgravity on cardiac electrical stability. The general hypothesis being tested in this specific aim is that there is an alteration in cardiac electrical activity as a result of exposure to simulated microgravity, resulting in increased susceptibility to ventricular arrhythmias.
3. To test the effectiveness of midodrine at preventing orthostatic intolerance following exposure to simulated microgravity.

Specific Aims of the current project:

1. To test a panel of countermeasures to orthostatic intolerance in subjects intolerant to pre bed rest head-up tilt, in conjunction with the evaluation of CSI and other cardiovascular measures as a potential means of predicting orthostatic intolerance and countermeasure effectiveness.
2. To test in a 16 day head-down tilt bed rest study the effectiveness of the orthostatic intolerance countermeasure identified for each individual during pre bed rest screening, in conjunction with the evaluation of CSI and other cardiovascular measures as a means of predicting orthostatic intolerance and countermeasure effectiveness.
3. To utilize MTWA analysis, in older men and post-menopausal women, to measure the effects of a panel of countermeasures for reducing cardiac electrical instability, and to study the effects of these countermeasures on baseline orthostatic tolerance and closed-loop cardiovascular regulation as measured by CSI and other cardiovascular measures.
4. To test utilizing MTWA analysis in a 16 day head-down tilt bed rest study the effectiveness of the cardiac electrical instability countermeasure identified for each individual during pre bed rest screening.

Accomplishments

1. Demonstrated in randomized bed rest study in male subjects that midodrine is an effective countermeasure to the development of orthostatic intolerance after simulated microgravity.
2. We have completed a head-down tilt bed rest study of pre-menopausal women and the data are being processed. Preliminary data suggest that women are more orthostatically intolerant than men both pre and post bed rest and may require a larger dose of midodrine.

3. Demonstrated that CSI measures of closed-loop autonomic cardiovascular control are down-regulated during microgravity.
4. Development of CSI means of separately measuring sympathetic and parasympathetic responsiveness .
5. Application of new CSI measures of sympathetic and parasympathetic responsiveness to study of effects of bed rest and changes in posture.
6. Demonstrated that CSI measures of sympathetic and parasympathetic responsiveness associated with tilt tolerance.
8. Prediction of tilt tolerance after bed rest from pre bed rest CSI measures.
9. Developed CSI measures of arterial total peripheral resistance (TPR) baroreflex and cardiopulmonary TPR baroreflex.
10. Identified changes in arterial and cardiopulmonary baroreflexes with bed rest .
11. Validation of CSI measures by means of computer simulation and analysis of animal data.
12. Developed new technique for continuous cardiac output monitoring. Validated accuracy in animal studies. Important applications to cardiovascular research and space medicine. Important application for monitoring of patients at risk of hemorrhage in space and on earth. Patent applied for and commercialization discussions proceeding.
13. Developed new principal components approach to system identification
14. Demonstrated that orthostatic intolerance is associated with altered venous compliance.
15. We have demonstrated for the first time that head-down tilt bed-rest increases the incidence of microvolt T-wave alternans, indicating that microgravity affects cardiac repolarization processes in a manner which may increase susceptibility to life threatening ventricular arrhythmias.
16. To validate MTWA as a potential predictor of susceptibility to ventricular tachyarrhythmias during exposure to microgravity, we have analyzed MTWA in a wide variety of patient populations. Because of the striking reproducibility of the MTWA results in numerous clinical trials, the clinical use of this technology is rapidly growing.

Effects of Space Flight on Cardiovascular Stability (Flight Study): Richard Cohen, P.I.

Specific Aims

This study involves Cardiovascular System Identification (CSI) and Microvolt T-Wave Alternans (MTWA) measurements made pre and post flight in order to:

- 1) Establish which specific physiologic mechanisms are responsible for alterations in integrated hemodynamic behavior due to spaceflight.
- 2) Determine if pre-flight measures can predict susceptibility to post flight orthostatic hypotension in individual crew-members.
- 3) Determine if cardiac electrical function is altered after spaceflight.

Accomplishments

We have worked closely with the teams at Johnson Space Center to prepare the details protocols and extensive documentation for this project, and to obtain all necessary approvals. We have tested the data collection protocol in a dry run session. We have presented the protocol to the astronauts on STS-121.

Computational Models of Cardiovascular Function for Simulation, Data Integration and Clinical Decision Support: Roger Mark, P.I.

Specific Aims

1. Develop procedures and algorithms for automated model-based extraction of physiologically relevant information from multivariate data streams, particularly those relevant to countermeasure development for orthostatic intolerance.
2. Develop strategies for more systematic and effective model identification and application, i.e., for the adaptation of model structures and parameters to the characteristics of the available physiological data and to the intended uses of the model.
3. Incorporate and evaluate our computational modeling technology as a core component of an advanced hemodynamic monitoring system, for use in clinical monitoring and decision support, particularly in critical care settings.
4. Develop and thoroughly test a graphical user interface for our current cardiovascular model.

Accomplishments

1. Have demonstrated ability to estimate cardiovascular parameters from transient hemodynamic response to sudden orthostatic stress. Estimation algorithm was tested using synthetic noise-corrupted data from a computational cardiovascular model.
2. Have performed extensive sensitivity analyses on computational model. The results demonstrate that besides total blood volume, venous tone feedback is among the principal parameters affecting hemodynamic response to the upright posture.
3. Have begun exploring model reduction schemes to adapt model structure to the particular characteristics of hemodynamic data streams both from astronauts and from patients in intensive care.

Currently investigating parameter estimation methodology in the context of hemorrhage in intensive care patients.

Mechanisms of Post-Space Flight Orthostatic Intolerance (Flight Study): Janice Meck, P.I.

Specific Aims

The aims of this study are to: characterize the responses of adrenergic receptors in the venous system and on lymphocytes; to characterize changes in nitric oxide physiology before and after spaceflight; to determine if these changes are similar in the upper and lower extremities; and to relate changes in the parameters to the occurrence of postflight orthostatic hypotension.

Accomplishments

The experiment has been pitched to three crews and we have volunteers for every Shuttle flight that is on the 2005 flight manifest. We have refined the protocol, according to lessons learned from ongoing research in the laboratory and have final approval of the protocol from the JSC CPHS. We're just waiting for a flight. In the meantime we have started collecting some of the same measures during the ongoing bed rest study and preliminary results suggest that the hypothesis of the flight study is correct (there is vascular remodeling in the legs, but not in the arms).

A Soluble Guanylyl Cyclase Mouse Knock-out Model: Ferid Murad, P.I.

Specific Aims

This proposal defines the physiological effect of soluble guanylyl cyclase (sGC) on cardiovascular function in a mouse simulated microgravity model. The goal of the proposed studies is to generate a sGC knockout mouse model to elucidate the role of sGC in the cardiovascular system in physiological and microgravity conditions.

1. To produce *Lox*-targeted cardiac-specific α_1 -sGC knockout mice utilizing standard recombinant cloning and transgenic techniques. Mice will be mated with cardiac specific *Cre* mice (available through Michael Schneider at Baylor College of Medicine, Houston, TX) to obtain the double *Cre/Lox* cardiac-specific α_1 -sGC transgenic progeny and smooth muscle specific *Cre* mice (available through Franz Hofmann at Institut für Pharmakologie und Toxikologie, Munich, Germany) to obtain the double *Cre/Lox* smooth muscle specific α_1 -sGC transgenic progeny. To characterize the effects of myocardial-specific and vascular smooth muscle-specific sGC gene disruption on general appearance, ECG, arterial blood pressure, heart rate, cardiac Doppler measurements, embryonic development and histology of the heart in knockout mice versus wild type mice.

2. To determine the role of the sGC pathway deficiency on the development of orthostatic intolerance that occurs during re-adaptation to gravity, using the established tail-suspended rodent model to simulate the microgravity conditions. To determine the time course of changes in cardiovascular function induced by re-adaptation to gravity in knockout mice (myocardium-specific and vascular smooth muscle –specific) compared with wild type mice. The effects of gene disruption on ECG, arterial blood pressure, heart rate and cardiac Doppler measurements will be recorded immediately and once a day for 3 days following de-suspension in knockout

mice versus wild type mice. To study the cardiovascular responsiveness in knockout mice versus wild-type mice following de-suspension. Animals will be challenged with vaso- and cardio-active agents. Pharmacological drugs will be administered immediately following de-suspension and once a day for 3 days, as cardiovascular parameters are monitored.

Accomplishments

1. Homozygous Lox-targeted Betal sGC mice were obtained. In order to accomplish that, the Lox-Betal sGC targeted ES line generated in the previous year were introduced into mouse blastocysts and chimeric male mice were obtained. To identify animals with transmission of the targeted ES cells into the germ line, chimeric males were bred with wild type C57Bl/6 females. The F1 offsprings were genotyped and Lox-targeted heterozygous mice were identified. Homozygous mice with Lox-targeted Betal sGC gene were obtained by crossing of the identified heterozygous mice. Presently, the colony of 20 mice of **pTr341 Betal-sGC-Lox** strain is established at Animal Facility of Institute of Molecular Medicine.

2. In order to gain an advanced knowledge about regulation of sGC expression in human body we continued characterization of β_1 human sGC gene promoter region initiated previous year and identified CCAAT binding factor (CBF) as critically important factor in β_1 sGC expression. The resulting research accomplishments were published in PNAS paper.

3. We investigated the role of the heme moiety in the basal state of human sGC and generated the constitutively active heme-deficient sGC enzyme which could be a useful reagent for a gene transfer therapy, amelioration of chronic hypertensive conditions and screening for novel inhibitors and activators of sGC. The resulting research accomplishments were published in a PNAS paper.

Ultrasonic Bone Stimulation: Countermeasure to Orthostatic Intolerance: Chet Ray, P.I.

Specific Aims:

1. To determine if ultrasonic bone stimulation improves orthostatic tolerance during head-up tilt. Orthostatic tolerance will be determined during 60° head-up tilt in OI patients and in subjects following 1 day of bed rest. We **hypothesize** that orthostatic tolerance will be improved during UBS in these groups of subjects. If this hypothesis is true, this would be the first evidence that UBS of the mastoid might serve as an effective and simple countermeasure for OI following spaceflight.

2. To determine the mechanism responsible for improved orthostatic tolerance by ultrasonic bone stimulation. Because it is believed that UBS of the mastoid engages the otolith organs, MSNA will be measure before and after UBS in young, aged, and vestibular deficient subjects. We **hypothesize** MSNA will be increased during UBS in young subjects but attenuated and absent in aged and vestibular deficient subjects, respectively. These results would indicate that an important mechanism of UBS is the engagement of the vestibulosympathetic reflex.

Accomplishments

Published 15 papers – 9 articles related to orthostatic stress or vestibular– cardiovascular responses.

Collected substantial data that ultrasonic bone stimulation (UBS) increases MSNA and this increase is similar to head-down rotation (HDR) in subjects (n = 11).

Obtained new NIH grant on vestibulosympathetic reflex in humans.

Non-Adrenergic Mechanisms of Cardiovascular Deconditioning: Art Shoukas, P.I.

Specific Aims in our previous funded proposal (10/01/2000 to 06/25/2003) to NSBRI were:

1. SA-I To determine mechanisms of impaired stroke volume response (SV) in a rat model of micro-gravity.
SA-Ia. To determine the role of myocardial contractility and loading conditions in the impaired SV response.
SA-Ib. To determine the role of cardiac atrophy in cardiovascular de-conditioning
2. SA-II To determine molecular mechanisms of vascular (systemic and pulmonary arterial, and venous) hypo responsiveness in a rat model of micro-gravity.
SA-IIa. To determine the role of abnormalities in vascular smooth muscle Ca^{2+} influx/release and myo-filament Ca^{2+} sensitivity in vascular hypo-responsiveness
SA-IIb. To determine the role of the endothelium in vascular contractile hypo-responsiveness.
3. SA-III To test pharmacologic countermeasures based on mechanisms that impair both SV responses, and vascular hypo-responsiveness in a rat model of micro-gravity.
4. SA-IV Flight Testing of Rats on STS-107, Columbia.

Specific Aims in our currently funded project (10/01/2004 to 09/30/2008) which tests the hypothesis that the NO/cGMP pathway is upregulated in both the heart and vasculature of models of microgravity resulting in vascular and myocardial contractile hyporesponsiveness, as well as vascular and myocardial atrophy.

1. SA1: To determine the role of the endothelium and the NO/cGMP pathway in vascular hypo-responsiveness and remodeling in microgravity.
2. SA2: To determine the role of NO in modulating myocardial contractility and hypertrophy.
3. SA3: To determine whether upregulation of the NO/cGMP system results in decreased vascular and ventricular stiffness as measured by integrated cardiovascular function and ventricular-vascular coupling.
4. SA4: To test countermeasures aimed at inhibiting NO/cGMP pathways for their ability to enhance vascular tone and reduce vascular hyporesponsiveness, restore myocardial contractile reserve, prevent NO mediated anti-hypertrophic responses, and restore arterial and ventricular stiffness.

Accomplishments

1. We have demonstrated impaired CO responses to an orthostatic challenge in rats following HLU which recovers in ~60hrs.
2. We have shown impaired α_1 -AR and non- α mediated contractile responses in aorta of HLU animals.
3. We have demonstrated primarily impaired α_1 AR contractile responses in the femoral arteries of HLU rats and demonstrated that the vascular phenomenon observed is reversible.
4. We have observed α_1 AR specific abnormalities in mesenteric micro-vessel responses. We have demonstrated the critical role for the α_1 b AR subtype in mediating contractile responses necessary during orthostatic stress in mesenteric vessels.
5. We have observed a decrease in α_1 AR specific radio-ligand binding in aortic vessels from HLU animals.
6. In total body perfusion experiments we have shown that there is an increased venous capacitance and venous contractile hypo-responsiveness as one potential cause for impaired SV responses.

7. In isolated venous vessel experiments we have been able to show that there is an increase venous capacitance, as indicated by their pressure diameter relationships and the contractile properties of these vessels is diminished by either electrical stimulation or with nor-epinephrine.
8. We have finalized an external non-invasive mechanical prototype device that peristaltically pumps blood from lower extremities and abdomen towards the heart to maintain stroke volume and cardiac output during an orthostatic challenge. Provisional Patent Number: 60/440,314.
9. We have been able to show that there is a drastic reduction in cardiac reserve in HLU animals and that the ability of the baroreceptor reflex to increase contractility in nearly totally abolished.
10. We have been able to show an impaired contractile responsiveness which appears to be both endothelial independent and dependent.
11. We have shown there is a significant relaxation component contributing to venous tone in mesenteric veins, which could be inhibited as a countermeasure to orthostatic intolerance.
12. In pulmonary vessels we have demonstrated:
 - Enhanced responses to endothelial cell dependent vasodilator stimuli (Ach).
 - Decreased responses to endothelial cell dependent vasoconstrictor stimuli (PE, U4).
 - Up-regulation of endothelial cell eNOS and sGC expression.
13. Enhanced vasodilator responses to endothelial cell independent vasodilator stimuli (SNP). Our preliminary data supports the notion that NOS expression and function is upregulated in vascular beds in models simulating microgravity. NO function is regulated by alterations in expression (transcriptional), and by post-translational control through dynamic interaction with a number of proteins that form a signal transduction complex with NOS-3.
14. We have recently demonstrated that NO function is dependent on the spatial confinement of signaling with NOS-1 regulating SR Ca^{2+} cycling and enhancing contractility, and NOS-3 modulating L-type Ca^{2+} channels and depressing sympathetically mediated enhanced contractility. Thus, NO may have functionally opposite effects based on the NOS isoform, and spatial confinement of signaling. In addition, a novel beta-AR, the beta-3 AR has recently been demonstrated to be coupled to NOS-3 in cardiac myocytes. Furthermore, both deficiency of NOS-1, NOS-3, or beta-3 AR independently results in myocardial hypertrophy.

These accomplishments have allowed us to refine mechanisms, begin to test countermeasures, and bridge the gap between animal models and human subjects in our understanding of micro gravity induced orthostatic intolerance.

Effects of Microgravity on Renal, Endocrine and Volume-Regulatory Function: Gordon Williams, P.I.

Specific Aims

Previous study

- To investigate the influence of age on the pattern of renal sodium handling and the acute responsiveness of the RAAS following simulated microgravity exposure.
- To investigate the influence of gender on the pattern of renal sodium handling and the acute responsiveness of the RAAS following simulated microgravity exposure.
- To investigate the effects of the alpha-1 agonist midodrine, as a countermeasure against orthostatic intolerance following microgravity exposure in women.

- To investigate the effects of spironolactone in older men on the renal-endocrine responses to simulated microgravity, and on changes in myocardial electrical stability resulting from microgravity exposure.

Current study

- To investigate the use of pre-bed rest tilt-test as a means to screen countermeasures against orthostatic intolerance and assess the influence of these countermeasures on the renal and endocrine responses to orthostatic stress.
- To test the hypothesis that the countermeasure found to be most effective in Specific Aim 1, will increase orthostatic tolerance post-simulated microgravity and do so by modifying the renal and endocrine responses to orthostatic stress.

Accomplishments

During the past several years, we have addressed the subject of renal, cardiovascular and endocrine volume-regulating factors using a simulated microgravity protocol (sixteen day-head-down tilt bed rest) in a rigorously controlled environment (in-patient General Clinical Research Center with constant dietary intake, controlled activity, and regulated temperature, light exposure and sleep/wake cycles). Among the several observations that we have made on the forty individuals studied to date, the followings are key for the present proposal:

- Initial loss of urinary volume and sodium followed by reestablishment of sodium balance through activation of the renin-angio system;
- Continuous potassium loss;
- A dissociation between renin and aldosterone responses to simulated microgravity and up-right tilt;
- A decrease in autonomic function with a change towards a sympathetic-dominated system;
- Cardiac electrical instability;
- Subjects intolerant to head-up tilt pre-bed rest are intolerant post-bed rest, but the reverse is not true, suggesting individual susceptibility to orthostatic intolerance independent of simulated microgravity;
- Finally, in a randomized double-blinded trial, we observed that midodrine was an efficient intervention for the treatment of orthostatic intolerance following simulated microgravity.

IV. TEAM PLANS

This coming year each of the individual projects will plan to proceed with its own research plan as described in its research proposal. In addition we plan to continue to foster integration among projects by means of individual collaborations between investigators, teleconferences and team retreats.

We plan to continue to seek collaborations with other NSBRI teams and NASA investigators. In particular, we have initiated discussions of working with NASA's research program in radiation with the objective of developing projects that examine possible adverse effects of radiation on the cardiovascular system.

One limitation of the current program is that to date our bed rest studies have been limited to 16 days. We are interested in studying effects of longer term bed rest on the cardiovascular system. Dr. Ray and Dr. Cohen have begun working with the NASA bed rest project at Galveston to obtain data during the 60 day bed rest campaigns that are planned. The leader of the NASA bed rest project is Dr. Meck who is one of our team's principal investigators.

We also plan to continue to develop the new cardiovascular technologies described above. In particular, we are dedicated to facilitate the commercialization process for these technologies so that they can actually be used to help patients in space and on earth.

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

ANNUAL PROGRAM REPORT October 18, 2004

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Project 1: Optimizing Light Spectrum for Long Duration Space Flight

PI: George C. Brainard, Ph.D. (See address above under Associate Team Lead)

Project 2: Circadian Entrainment, Sleep-Wake Regulation and Performance during Space Flight

PI: Charles A. Czeisler, Ph.D., M.D. (See address above under Team Lead)

Project 3: Countermeasures to Neurobehavioral Deficits from Partial Sleep Loss

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Project 4: The Role and Characterization of Novel Photoreceptor Mechanisms Regulating Circadian rhythms, Sleep, Body Temperature and Heart Rate: Implications for Creating Artificial Light Environments in Space.

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Project 5: Primate Circadian Rhythms in the Martian Environment

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Project 6: Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure

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Project 7: A Model of Circadian Disruption in the Space Environment

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Project 8: Circadian and Vestibular System Relationships

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Project 9: Long-term Exposure to Dim Light Desynchronizes the Circadian System of Rat

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Project 10: Animal Model for Sleep Loss and Circadian Disruption

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Date

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I. ABSTRACT

The success of human space missions depends on each astronaut remaining alert and vigilant while operating sophisticated equipment and following complex procedures. During long-duration space flight, the space environment affects a number of physiological systems critically involved in human performance, and it is vital to mission success to understand the biological limits of human performance under space flight conditions. This team is focused on these issues and, in particular, is concerned with the following aspects of the space environment: microgravity, altered light-dark cycles, altered or reduced sleep/rest opportunities, high levels of automation, and habitation in a remote, inaccessible location. The primary thrust of this team's research program involves altered circadian organization, sleep disruption and cumulative sleep loss, and the associated neurobehavioral decrements occurring during long-duration space flight.

The goals of the Human Performance Factors, Sleep and Chronobiology (HPFSC) Team are to: (1) Characterize and quantify the adverse effects of long-duration space flight on sleep and circadian rhythms; (2) Characterize and quantify the effect of sleep loss and/or circadian dysfunction on physical and neurobehavioral performance; (3) Understand the basic mechanisms underlying the deterioration of sleep, circadian organization and human neurobehavioral function during space flight; (4) Develop high-fidelity mathematical models of performance based on circadian organization and sleep-wake history; (5) Develop effective countermeasures to optimize sleep and facilitate circadian adaptation in the space environment and thereby maintain optimal neurobehavioral performance; (6) Develop new methods for monitoring the status of sleep, sleep homeostasis, circadian rhythmicity, light exposure and neurobehavioral performance during space flight, with possible spin-off applications on Earth.

The team research objectives are driven by the Critical Path Roadmap (2000) related to Human Performance Failure because of Sleep and Circadian Rhythm Problems. The current research program involves ten ground-based research projects. Many of the projects impinge on more than one critical risk within CPR Risk Area 19 (Critical Questions #s 6.05, 6.06, 6.07, 6.08, 6.18, 6.21) and CPR Risk Area 20 (Critical Questions #s 6.11, 6.12, 6.15). The strategy of the Human Performance Factors, Sleep and Chronobiology Team is to develop a synergistic interaction between research projects at the molecular, cellular, organismic, and human levels, and to integrate predictive biomathematical modeling of the sleep and circadian systems. The resultant research and team interactions are intended to accelerate progress on countermeasures that reduce the risk of human neurobehavioral or physiological performance failure during space exploration. Specific countermeasures under study include lighting, napping, scheduling and non-photic interventions such as meal-timing, exercise, and melatonin administration.

II. INTRODUCTION

The need for sleep and an entrained circadian pacemaker have a sustained influence over many biomedical systems essential for maintaining astronaut physical condition, mental health, and performance capability. Dysfunction of sleep and circadian systems can adversely affect an organism's ability to respond to environmental challenges and has been linked to physiological and psychological disorders. The success of human space missions depends on each astronaut remaining alert and vigilant while operating sophisticated equipment and following complex procedures. During exploration class space missions, the space environment affects a number of physiological systems critically involved in human performance, and it is vital to mission success to understand the biological limits of human performance under such conditions. It has been demonstrated that both acute gravitational changes and space flight disrupt circadian rhythms and reduce sleep. Since circadian disruption and sleep loss result in both physiological and performance deficits, this team is focused on these issues and, in particular, is concerned with the following aspects of the space environment: microgravity, altered light-dark cycles and altered or reduced sleep/rest opportunities that may involve extended durations of wakefulness. The primary thrust of this team's research program involves altered circadian organization, sleep disruption and cumulative sleep loss, and the associated neurobehavioral decrements occurring during exploration class missions. This area has a high degree of relevance to a number of cardiovascular and immune changes, neurovestibular alterations and nutritional needs, and behavioral and psychological health in space flight.

The Human Performance Factors, Sleep and Chronobiology Team's program addresses risks and hazards in space flight that have been identified in the Human Behavior and Performance Discipline Area of the Critical Path Roadmap Baseline Document (2000). Specifically:

- Human Performance Failure Because of Sleep and Circadian Rhythm Problems (19)
- Human Performance Failure Because Of Human System Interface Problems and Ineffective Habitat, Equipment, Design, Workload, or In-flight Information and Training Systems (20)

Our team has the following three **risk-based goals** for its program:

Goal 1: *Reduce the risk of human neurobehavioral or physiological performance failure due to disruption of circadian phase, amplitude, period, or entrainment during space exploration.*

Goal 2: *Reduce the risk of human neurobehavioral or physiological performance failure due to acute or chronic degradation of sleep quality or quantity during space exploration.*

Goal 3: *Reduce the risk of human neurobehavioral or physiological performance failure due to habitat design, equipment design or workload distribution during space exploration.*

The Human Performance Factors, Sleep and Chronobiology Team is focused on developing countermeasures for the sleep loss and circadian dysfunction and associated neurobehavioral and physiological performance decrements that occur during long-duration space flight. These countermeasures may include behavioral, pharmacological, environmental light or other adaptive approaches such as meal timing to maintain function and performance under the adverse conditions of exploration class space missions.

III. RESEARCH PROGRAM STRUCTURE AND DESIGN

The Human Performance Factors, Sleep and Chronobiology Team is focused on developing countermeasures for the sleep loss and circadian dysfunction and associated neurobehavioral performance decrements that occur during long-duration space flight. The team research objectives are driven by the Critical Path Roadmap related to Human Performance Failure because of Sleep and Circadian Rhythm Problems. The current research program involves nine ground-based research projects. The strategy of the Human Performance Factors, Sleep and Chronobiology Team is to develop a synergistic interaction between research projects at the molecular, cellular, organism, and human levels, and to integrate predictive biomathematical modeling of the sleep and circadian systems.

Over the past year, the LHPFSC has been comprised of ten PIs. In order to achieve the goals listed above, the Human Performance Factors, Sleep and Chronobiology Team has identified the following six interrelated themes within this research area:

- A. **Effects of long-duration space flight on sleep and/or circadian rhythmicity.** The focus of this theme is to identify and understand the mechanism underlying the effect of long-duration space flight (microgravity, altered light intensity, loss of geophysical cues, isolation, altered physical activity, etc.) on neurobiologic, endocrinological, and behavioral functions (molecular, cellular and organismic) that control sleep and circadian systems.
- B. **Effects of sleep loss and/or circadian dysfunction on physical and neurobehavioral performance.** The focus of this theme is to identify and to understand the mechanisms underlying the acute and chronic adverse effects that sleep loss, sleep disruption, and/or circadian dysfunction have on critical physiologic and performance parameters during long-duration space flight (e.g., neurophysiologic function, physiological alertness, vigilance, cognitive performance, mood/morale, problem solving and communication).
- C. **Predictive modeling of performance based upon circadian organization and sleep homeostasis.** This theme is concerned with the development of analytical or phenomenological mathematical models that predict individual human performance capability by involving multiple subsystems (e.g., circadian rhythmicity, sleep homeostasis, work-rest schedules, etc.) as an integrated unit across levels of organization, and by estimating the impact of countermeasure use designed to optimize human physical and/or neurobehavioral performance.
- D. **Countermeasures to optimize sleep and facilitate circadian adaptation in space and maintain optimal neurobehavioral performance.** The research program of this team will not only define the impact of the space environment on sleep and circadian rhythmicity and the effects of the sleep loss and circadian dysfunction on performance but also will develop methods to counter the adverse physiological and behavioral events. These countermeasures may include behavioral, pharmacological, environmental light or other adaptive approaches to maintain function and performance under the adverse conditions of long-duration space flight.
- E. **Monitoring and assessment during space flight.** This theme deals with the development of methods for monitoring the status of sleep, sleep homeostasis and circadian organization, as well as technologies that monitor ambient lighting conditions on space shuttle and ISS and assess and update the current functional status or performance capability of the individual

The Human Performance Factors, Sleep and Chronobiology Team is focused on developing countermeasures for the sleep loss and circadian dysfunction and associated neurobehavioral and

physiological performance decrements that occur during long-duration space flight. The research program for the Human Performance Factors, Sleep and Chronobiology Team research has involved ten research projects that collectively address the five research themes described above. The schematic of the circadian and homeostatic regulation of sleep and alertness and physiological functions shown in Diagram 1 illustrates the relationships between the ten current ground-based experiments that comprise the NSBRI Human Performance Factors, Sleep and Chronobiology Team, with the principal targets of each project indicated. This diagram illustrates the interrelated nature of these projects, designed to fill critical gaps in knowledge that need to be filled in order to develop effective countermeasures for long-duration space flight. Each of the individual projects is summarized below and in Table 1, including which goal(s) are addressed and countermeasure targets.

Brainard et al.: Optimizing Light Spectrum for Long Duration Space Flight

The physiological changes caused by disturbed circadian rhythms and altered sleep-wake patterns can result in decrements in alertness, concentration, and performance. This project addresses these risk factors, which threaten the safety of personnel and the objectives of space missions as stated in Goals 1 and 3.

Countermeasure targets include:

1. Identification of the optimum spectrum for photic entrainment and resetting of the circadian pacemaker.
2. Design specifications for space suit visors and the windows used in space vehicles and habitats;
3. Engineering parameters for the ideal spectral distribution for illumination of general living quarters during space exploration.

Czeisler et al.: Circadian Entrainment, Sleep-Wake Regulation & Performance during Space Flight

The intent of this project is to develop countermeasures to facilitate adaptation of the human circadian pacemaker to the 24.65-h day length of Mars, which is outside the range of entrainment of the human circadian pacemaker given the weak synchronizing stimuli within the Martian habitat. This project applies to Goals 1, 2 and 3.

The primary *countermeasure target* is to evaluate the efficacy of intermittent bright light pulses as a treatment to reduce the risk of the misalignment of circadian phase, sleep disruption, associated decrements in neurobehavioral performance and reduction in nocturnal growth hormone secretion experienced by individuals exposed to the 24.65h Martian day.

Dinges et al.: Countermeasures to Neurobehavioral Deficits From Partial Sleep Loss

Using a response surface experimental paradigm (RSM), this project seeks to reduce neurobehavioral deficits and fatigue due to inadequate sleep in astronauts by investigating how variations in sleep duration and its circadian placement relate to the return of performance per time invested in sleep. This project applies to Goals 2 and 3.

Countermeasure targets include determination of the amount of naptime necessary to compensate for reduced nocturnal sleep periods for the prevention of cumulative sleepiness and performance deficits.

Foster et. al.: Novel Photoreceptor Mechanisms Regulating Circadian Rhythms, Sleep, Body Temperature and Heart Rate.

The results from these experiments will be integrated with studies on human subjects undertaken by other team members (particularly Project 1) and allow us to understand how the classical rod and cone visual system interacts with the non-rod, non-cone ocular photoreceptors to regulate physiology and behavior. Space flight has been associated with abnormal circadian rhythms, sleep-wake patterns, mood, concentration, and alertness.

Countermeasure targets include the design of new lighting sources that are either highly effective in regulating these novel photoreceptors or leave them largely unstimulated. This project applies to Goals 1 and 2.

Fuller et al.: Primate Circadian Rhythms in the Martian Environment

This project is focused on the ability of the circadian time system to synchronize to the Martian photic environment (spectrum and period) by examining the effects of 1.0, 1.5 and 2.0G on the period of the circadian pacemaker. A G vs. period model will be developed to predict the effect of the 0.38 G Martian environment on the period of the circadian pacemaker. Long-term physiological and behavioral responses will be examined.

Countermeasure targets include the use of timed bright light pulses on circadian entrainment. This program will develop a primate model to evaluate physiological and behavioral consequences of long-term exposure of males and females to altered lighting and gravitational environments. This project applies to Goals 1 and 3.

Klerman et al.: Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure

The intent of this project is to further develop and refine our mathematical dynamic stimulus processing model so that it can accurately predict the phase and amplitude of the human circadian system under any lighting system especially those which are present in space. The mathematical Neurobehavioral Performance model validated against performance data collected will result in the development of a user-friendly Performance Simulation Software program. This project applies to Risk-based goals 1 through 3 and Non-Risk-Based Goal 4 (see below).

Countermeasure targets include the design of shift schedules to allow astronauts to receive available bright light at appropriate times for proper circadian alignment with their sleep/wake schedules.

Menaker et al.: A Model of Circadian Disruption in the Space Environment

This project proposes to evaluate the effects of "constant" conditions and shift work schedules on the maintenance of circadian rhythmicity when the central and peripheral structures are abnormally phased. The resulting abnormal circadian organization is "dysphasia." This project applies to Goal 1.

Countermeasure targets include an evaluation of meal timing, melatonin administration, forced exercise, and short pulses of complete darkness as a treatment to reduce the risk of circadian dysphasia.

Morin et al.: Circadian and Vestibular Relationships

This project seeks to determine the route by which a correlate of the non-photoc stimulus, i.e., locomotion, might gain access to the circadian rhythm system and shift rhythm phase. It has also opened the possibility that the vestibular system is a specific route by which sensory information related to head movement might gain access to the circadian system. This project applies to Goal 1.

Countermeasure targets include an evaluation of a non-locomotor, non-photoc three-dimensional motion stimulus to activate functionally the vestibular and circadian systems, laying the

groundwork for the future development of novel approaches for the treatment of space motion sickness and for resetting circadian phase.

Tosini et al.: Long-Term Exposure to Dim Light Desynchronizes the Circadian System of Rats

The goal of this project is to understand the mechanisms responsible for the desynchronization of circadian rhythm in locomotion and the enzymes responsible for the production of melatonin. Investigating the effect that internal desynchronization has on the immune response and motor and cognitive performances. This project applies to Goal 1.

Countermeasure targets include an evaluation of the use of melatonin as a pharmacological agent to counteract desynchronization of the circadian rhythms.

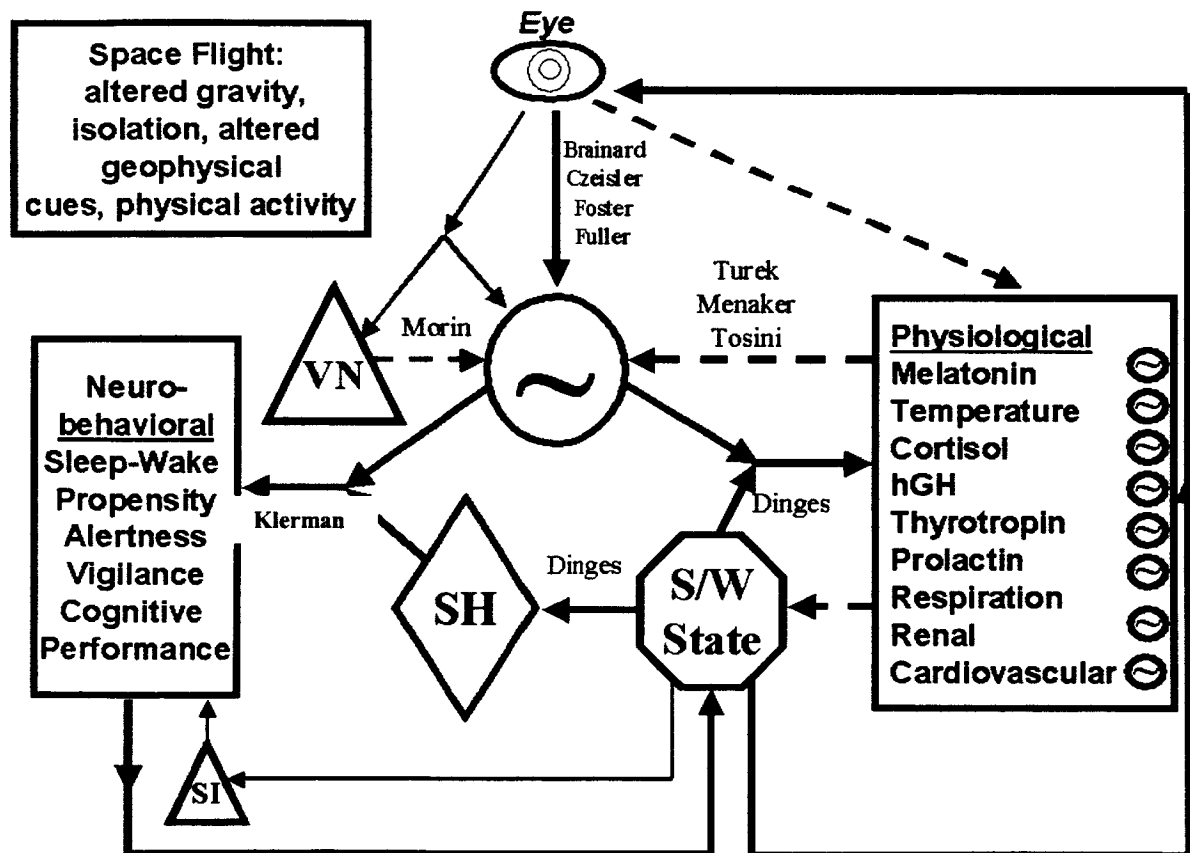
Turek et al.: Animal Model for Sleep Loss and Circadian Disruption

This project will focus on determining the effects of 12 hours of imposed wakefulness on circadian rhythms, sleep-wake cycles, neurobehavioral and motor performance measures during normal active and inactive periods. This project applies to Goals 1 and 2.

Countermeasure targets include treatment exercise and with either physiological or pharmacological dose of melatonin reduce the effects of circadian disruption and sleep loss as well as alleviate the adverse effects associated with work at different times of day.

During the past year, nine of these ten projects completed three-year funding cycle. The NBRI issued an open solicitation to select both new and continuing projects for support through a competitive peer-review evaluation process. Six of these nine projects were selected for funding. (Pis: Drs. Brainard, Czeisler, Dinges, Klerman, Menaker, and Tosini); these six projects, together with one continuing project that began this past year. (PI: Dr. Foster), will comprise the HPFSC Team over the next 3-4 years. In 2004, there was also a competitive re-evaluation of the leadership of the HPFSC Team. After evaluating applications submitted in response to an open solicitation, the NSBRI Board of Directors selected Dr. Charles Czeisler to serve as Team leader and Dr. George Brainard to serve as Associate Team Leader of the HPFSC Team for the next four years.

Diagram 1. Description of (2004) Program for Human Performance Factors, Sleep and Chronobiology. This diagram illustrates the relationships between the different physiological systems investigated by the different projects on the team. Illustrated is the influence of the retinal light exposure on the human circadian clock (circle with the oscillator symbol ~) and the influence on the sleep-wake state (S/W), and their effect on a number of physiological variables (melatonin, temperature, etc.). A combined influence of the circadian clock and sleep-wake is exerted on neurobehavioral variables (sleep-wake propensity, alertness, etc.). The sleep-wake state influence is illustrated via the intermediary of the sleep homeostat (SH), and sleep inertia (SI). The global influence of factors associated to Space Flight (micro gravity, isolation, etc.) on the sleep and circadian systems is also represented. The interaction of the Vestibular Nucleus (VN) and its output pathways with the circadian pacemaker is being investigated by one project.



HUMAN PERFORMANCE FACTORS, SLEEP AND CHRONOBIOLOGY PROGRAM

Table 1. Project Research Activities

PI/Project	Risk(s) Addressed	Countermeasure Target	Experimental System	Phase 1 Activities: Focused Mechanistic Research	Phase 2 Activities: Preliminary Countermeasure Development Research	Phase 3 Activities: Mature Countermeasure Development Research
BRAINARD /Optimizing Light Spectrum for Long Duration Space Flight	Goals 1, 3	Optimum light spectral distribution	Healthy male and female human subjects	Develop melatonin fluence-response curves below 440 nm and above 600 nm in human subjects. Develop action spectrum between 400 and 700 nm in subjects with undilated pupils	Identification of optimum light spectral characteristics for maintaining or adjusting circadian phase and sleep-wake cycle in astronauts and ground control workers. Preliminary test of monochromatic stimuli for phase shifting human circadian rhythms	Preliminary development and testing of prototype fluorescent lamps for pre-launch and in-flight use as lighting countermeasure. Assist in designing a novel light panel for circadian stimulation. Assist in developing protocols for comparing head mounted light therapy devices
CZEISLER /Circadian Entraining, Sleep-Wake Regulation & Performance During Space Flight	Goals 1, 2, 3	Blue enhanced ambient light	Healthy male and female human subjects scheduled to non-24-hour day lengths in an environment shielded from periodic, 24-h time cues	Quantification of the intrinsic period and the limits of entrainment of the human circadian pacemaker; investigation of the effect of circadian misalignment on sleep, neurobehavioral performance and neuroendocrine function	Preliminary evaluation of the efficacy of intermittent bright light pulses on circadian entrainment to non-24-hour work-rest schedules, as required on Mars	Full-scale clinical trial of age and gender matched astronaut surrogates living for extended durations on a non-24-hour work schedule while exposed to intermittent bright light at the most effective wavelength
DINGES /Countermeasures to Neurobehavioral Deficits from Partial Sleep Loss	Goals 2 and 3	Naps and split sleep schedules	Healthy male and female human subjects	Mathematically track neurobehavioral performance deficits associated with chronic sleep restriction. Examine sleep efficiency and architecture during restricted sleep periods at different circadian phases	Develop response surface map paradigms to further understand the interaction between sleep duration, sleep-wake placement and neurobehavioral functioning	Development of optimal sleep-wake schedules (including main and supplementary sleep episodes) to ensure maintenance of high level neurobehavioral functioning

FOSTER/Novel Photoreceptor Mechanisms Regulating Circadian rhythms, Sleep, Body Temperature and Heart Rate.	Goals 1 and 2	Mechanisms and role of novel ocular photoreceptors.	Normal and transgenic mouse models	Telemetry of body temperature, heart rate, EEG. Fos expression in the VLPO of <i>rd/rd</i> <i>cl</i> and normal mice. Initiate Proteomics and microarray projects to identify genes and proteins associated with novel photoreception.	Action spectroscopy of non-rod, non-cone responses to light. Tract-tracing of non-rod, non-cone photoreceptive pathways. Primary analysis of genes and proteins associated with novel photoreception.	Advanced analysis of genes and proteins associated with novel photoreception including the development of transgenic mice to place candidate genes into a functional context.
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Note:

- Goal 1:** Reduce the risk of human neurobehavioral or physiological performance failure due to disruption of circadian phase, amplitude, period, or entrainment during space exploration.
- Goal 2:** Reduce the risk of human neurobehavioral or physiological performance failure due to acute or chronic degradation of sleep quality or quantity during space exploration.
- Goal 3:** Reduce the risk of human neurobehavioral or physiological performance failure due to habitat design, equipment design or workload distribution during space exploration.

FULLER/Primate Circadian Rhythms in the Martian Environment	Goals 1 and 3	Bright light pulses	Rhesus monkeys as human surrogates. Large-diameter centrifuge to produce altered environment. Controlled lighting period, intensity and spectra. Long duration exposure in controlled animal facilities.	Determine the effect of altered gravity on primate circadian rhythms, principally the endogenous clock period.	Enhance entrainment response to low light (ISS, Martian habitat), reddish light (Mars), and non-24 hour schedules by means of exposure to light pulses. Definition of bright light source for light pulses. Studies will address timing and efficacy of bright light exposure.	Projected application of bright light pulses to prevent loss of circadian entrainment, sleep and rhythm disturbances, performance decrements.
JEWETT/Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure	Goals 1,2 and 3	Design of rest/work and sleep/wake schedules	Previously collected human data;	Determine the nature of amplitude recovery dynamics of human circadian system.	Design shift and sleep schedules for proper circadian alignment. Validate refined circadian amplitude dynamics of light model with data from new human phase shifting experiments	Incorporate refined light model into circadian components of neurobehavioral performance model and predict the performance in human phase shifting experiments
MENAKER/ A Model of Circadian Disruption in the Space Environment	Goals 1	Coupling between multiple circadian oscillators	Transgenic rat incorporating a circadian luciferase reporter gene	Description of system disintegration under simulated space flight conditions	Repair system disintegration with timed application of light, food and melatonin	Transfer working countermeasures to humans
MORIN/Circadian and Vestibular Relationships	Goals 1	Anatomical & functional issues linking the vestibular & circadian systems	Anatomical tract tracing using retro and anterograde transport of labels	Understanding the basic anatomical & functional pathways linking vestibular & circadian	N/A	N/A

				Study of brain regions for stimuli responses known to alter vestibular functions Phase shifts in circadian rhythms	systems		
TOSINI/Long-term Exposure to Dim Light Desynchronizes the Circadian System of Rats	Goal 1	Coupling between central and peripheral oscillators	Measuring expression of gene in peripheral tissues	Identification of the effects on the circadian system of prolonged exposure to constant conditions	Use of melatonin as synchronizing agent		
TUREK/Animal Model for Sleep Loss and Circadian Disruption	Goals 1 and 2	Exogenous melatonin administration and exercise	Two strains of mice exposed to 12 hours of forced activity each day for 10 consecutive days	Determine the effects of 10 days of chronic partial sleep loss on sleep/wake, circadian rhythms & performance	Testing the effectiveness of countermeasures (melatonin, exercise) using a mouse model	Transfer working countermeasures to humans and provide data to refine mathematical models	

In addition to the focus of the Human Performance Factors, Sleep and Chronobiology Team on achieving Risk-based Goals, there are also a number of important, **non-risk based goals** that the team is targeting, as follows:

Goal 4: *Develop new methods for monitoring the status of sleep, sleep homeostasis, circadian rhythmicity and neurobehavioral performance during space flight.*

To achieve this goal, current studies are being conducted to assess the potential of using the Actiwatch-L (a wrist-worn light and actigraphy recording device already approved for space flight) to monitor sleep and light exposure of individual crew members while in space. This device could replace more extensive polysomnography devices used in more recent studies of sleep in space. Studies are also underway that compare the wrist-level Actiwatch-L light recordings with eye-level light measurements. Work is progressing on the use of the Actiwatch-L measurements as inputs to a mathematical model that can then predict the level of sleep homeostasis, phase of circadian rhythmicity and relative neurobehavioral performance levels.

Goal 5: *Develop new methods for monitoring ambient and retinal light exposure (illuminance/photopic lux, broadband visible irradiance, and circadian effective illuminance/circadian lux) on board space shuttle and ISS during space flight and on planetary habitats.*

For measurement of retinal light exposure in space, please see Goal 4 above. For ambient light exposure, wall-mounted ambient light recording devices have been tested aboard the Space Shuttle in the Neurolab flight. The team's current studies will help determine the circadian effective illuminance and irradiance levels, and then these recording devices can be refined to measure circadian-activating light levels more precisely.

Goal 6: *Develop Earth-based applications of technologies for non-invasively monitoring the status of sleep, sleep homeostasis, circadian rhythmicity and neurobehavioral performance for industrial and medical use.*

The polysomnography device that was developed for the recording of sleep in space in the Neurolab Shuttle flight have become a useful, wire-free device for recording polysomnography in lab-based and home-based basic science and clinical studies. This technology has the advantage of being appropriate for use when ambulatory, and straight-forward enough for a trained person to apply to themselves.

The use of salivary melatonin as a marker of circadian phase has been applied in both space and on Earth and is a technology that allows the validation of experimental and modeling results in field studies in which plasma melatonin measurements would not be possible.

Mathematical models that are developed to predict neurobehavioral performance in space are also being used to determine appropriate shift scheduling, light exposure, sleep timing, and countermeasure applications for shift workers, pilots, military and medical personnel, and transportation workers who also face the challenges of restricted sleep and circadian misalignment here on Earth. Neurobehavioral test batteries that are developed for these projects are useful for the validation of mathematical models in field and laboratory studies as well.

Goal 7: *Develop Earth-based applications of high-fidelity mathematical models of performance based on circadian organization and sleep-wake history for industrial and medical use.*

The mathematical models of performance that are being developed in this project can be applied to any Earth-based situation in which it would be helpful to know the effects of a sleep/wake schedule and a light exposure pattern on resulting neurobehavioral performance (e.g., shift workers, pilots, military and medical personnel, and transportation workers). Therefore, the mathematical models developed here have been programmed into user-friendly simulation software that can be used by anyone to predict neurobehavioral performance given light exposure levels and sleep/wake history. This software is updated with model revisions and user-interface improvements on a regular basis.

Goal 8: *Develop Earth-based applications of technologies developed to reduce the risk of human neurobehavioral or physiological performance failure due to disruption of circadian phase amplitude, period or entrainment.*

The studies conducted here improve our understanding of the effects of light on the human circadian system, and the role that the circadian system plays in neurobehavioral performance. These findings are incorporated into our mathematical models on an ongoing basis. This allows us to then determine the best light schedule and intensities to reduce the risk of performance failure by appropriately aligning the circadian system with the work/rest schedule. This technology is already currently in use in transportation, military and industrial settings here on Earth.

Goal 9: *Develop Earth-based application so technologies developed to reduce the risk of human neurobehavioral or physiological performance failure due to acute a chronic degradation of sleep quality or quantity.*

Our projects will help determine the amount and timing of sleep that best allows people to work extended and/or misaligned shifts with the least risk of performance failure. These findings will also be incorporated into the mathematical model being developed here. The model can then be used to help schedule rest/nap/sleep times so that they are the most effective in improving performance levels.

Goal 10: *Develop Earth-based applications to technologies developed to reduce the risk of neurobehavioral or physiological performance failure due to extended duration work schedules (e.g., on-call schedules used in medical training, nuclear power plant shutdowns, military operations) or night shift work.*

Studies investigating the effects of extended duration work schedules in these projects allow us to determine the best timing of countermeasures (light exposure, naps, melatonin, etc.) to improve performance. These findings are completely applicable to any extended duration work schedules used here on Earth.

Goal 11: *Integrate research and analysis*

Our goal is to integrate research within the Human Performance Factors, Sleep and Chronobiology Team, with other teams, and with work being done by Team investigators not directly supported by NSBRI.

IV. RESEARCH PROGRAM ACCOMPLISHMENTS

The program accomplishments of each of the individual projects is summarized below:

Project 1: Optimizing Light Spectrum for Long Duration Space Flight

PI: George Brainard, Ph.D.
Thomas Jefferson University

Research Focus: Develop and improve pre-launch and inflight lighting countermeasures by identifying optimal spectral transmission characteristics for visors and windows, and engineering the ideal spectral distribution for illumination of living quarters.

Specific aims:

Test the hypotheses that:

1. Wavelengths of light below 440 nm and above 600 nm are active in regulating melatonin secretion via measurement of fluence response curves in humans;
2. There will be a loss of sensitivity to monochromatic light when the eyes are not pharmacologically dilated during the melatonin suppression test;
3. There will be a shift in spectral sensitivity of light regulation of melatonin secretion when the eyes are not pharmacologically dilated.

Research Progress 11/1/2003-10/31/2004:

I. EXECUTIVE SUMMARY

Risk factors for the health and safety of astronauts and NASA ground control workers include disturbed circadian rhythms and altered sleep-wake patterns. These physiological changes can result in decrements in alertness, concentration, and performance, all of which threaten the safety of personnel and the objectives of space missions. In studies of astronauts and NASA ground control workers, light treatment has been used as an effective countermeasure to provide entrainment of sleep-wake patterns and other circadian rhythms. It is important to optimize light as a countermeasure for circadian and sleep disruption in space flight missions. For civilians, light treatment is being tested for improving circadian entrainment and enhancing both performance and alertness in shift workers. A Congressional report estimates that 20 million full time workers in the United States are shift workers and that they have increased health problems including higher risk of cardiovascular disease, gastrointestinal distress, as well as cognitive and emotional problems. The long term goal of our research is to determine the best wavelengths of light for use as a countermeasure during long duration space flight, as well as for adjusting circadian and sleep disruption in civilians.

Data from the specific aims can be used to improve light treatment as a countermeasure

for circadian and sleep-wake disruption in NASA space flight missions, identify the best spectral transmission for space suit visors and the windows used in space vehicles and habitats, and engineer the ideal spectral distribution for illumination of general living quarters during space exploration.

II. RESEARCH ACTIVITY

Relevant to Specific Aim 1, several studies have been completed within the past research year. Eight subjects have now completed a melatonin suppression study using monochromatic 630 nm light for a total 60 subject/study nights, 29 within the past year, across 8 different light conditions. Another 8 subjects have started and completed a similar study using short wavelength monochromatic light at 405 nm for a total of 16 subject/study nights. Furthermore, a study of polychromatic long wavelength light was designed, started, and completed within the past research year that totaled 37 subject/study nights across 4 conditions. The latter study is also the subject of a graduate thesis by a member of the laboratory.

Work on Specific Aim 2 was completed before the start of the past project period, but data continues to be analyzed in preparation for a manuscript on quantifying the relative effects of pharmacological pupil dilation on melatonin suppression, and for practical application during space flight.

Experimental work on Specific Aim 3 was completed within the past research year. Seven subjects completed the final 21 subject/study nights of equal photon density exposures of different wavelengths of monochromatic light for a total of 63 subject/study nights over 9 different conditions. This data is in the process of being analyzed in order to understand if there is a shift in the peak sensitivity of melatonin suppression if the pupils are not pharmacologically dilated.

A total of 103 subject/study nights related specifically to this NSBRI funded project have been completed within the past research year. This required the recruitment, screening, and running of experiments, with at least one week of rest for each subject between study nights. Analysis of data and preparation of manuscripts is ongoing for both this work and work performed in previous research years. An opportunity to accelerate progress toward an operational lighting countermeasure required diverting some efforts away from our original specific aims. We understood from interactions with NSBRI headquarters that working toward a "deliverable" optimized lighting countermeasure was indeed desirable. Consequently, substantial progress was made this year on the set up, design, and initiation of experiments that relate to the "blue-enriched" polychromatic light that theoretically will provide a stronger circadian stimulation but also support vision. In collaboration with the HPFSC team, Philips Lighting, B.V. has designed, built, and shipped prototype fluorescent lamps for this purpose. A pilot study was completed on 4 healthy subjects probing the melatonin suppressing capacity of one lamp prototype. This study involved 12 subject/study nights. Subsequently, at Thomas Jefferson University a new laboratory has been set up for testing several lamp prototypes over the next four years. This facility is now fully operational.

III. KEY FINDINGS AND IMPLICATIONS FOR FUTURE RESEARCH

A key finding in the past research year is that light in the long-wavelength portion of the spectrum, even at very high intensities, is relatively weak in its capacity to suppress melatonin. This is now well established for monochromatic light at 630 nm, in which 7 healthy male and female subjects were exposed to 8 different intensities and a control night. Furthermore, work with long-wavelength polychromatic light above 650 nm now completed using 8 healthy male and female subjects suggests that this too is very weak in its capacity to suppress melatonin. These findings support the hypothesis for our next phase of research: that polychromatic light with an emphasis in the blue portion of the spectrum will be more efficacious in its ability to suppress melatonin. These data also will guide models of how to use the ambient daylight on Mars for circadian adaptation of astronauts. The use of polychromatic light sources for future research will not only be critical to elucidating our understanding the underlying physiology of the effects of light on the human circadian system, but will also prove potentially very valuable to the specific needs of astronauts as a countermeasure to sleep and circadian disruption in space flight.

PUBLICATIONS

Peer-Reviewed:

Fucci, Robert L., James Gardner, John P. Hanifin, Samar Jasser, Brenda Byrne, Edward Gerner, Mark Rollag, and George C. Brainard. Toward optimizing lighting as a countermeasure to sleep and circadian disruption in space flight. *Acta Astronautica* (in press).

Brainard, George C., and Gena Glickman. The biological potency of light in humans: significance to health and behavior. *Light and Engineering*, Vol. 12, No. 1 (2004).

Abstracts:

Byrne, B., G. Glickman, C. Pineda, and G.C. Brainard. Light therapy for seasonal affective disorder with 470 nm narrow-band light-emitting diodes. 16th Annual Meeting for Light Treatment and Biological Rhythms, May 2004.

Glickman, G., J. Hanifin, R. Fucci, M. Rollag, E. Gerner, B. Byrne, S. Jasser, J. Gardner, and G.C. Brainard. Monochromatic light-induced melatonin suppression in humans with freely reactive pupils: potential application to light therapy. 16th Annual Meeting of the Society for Light Treatment and Biological Rhythms, May 2004.

Brainard, G.C., D. Sliney, J. Hanifin, G. Glickman, B. Byrne, J. Greeson, S. Jasser, E. Gerner, S. Wengraitis, and M. Rollag. Phototransduction for the human retinohypothalamic tract: high sensitivity to short wavelength light. 9th Annual Meeting of the Society for Research on Biological Rhythms, June 2004.

Lockley, S.W., E.E. Evans, G.C. Brainard, C.A. Czeisler, and D. Aeschbach. Wavelength dependent effect of light on vigilance and performance. 17th Congress of the European Sleep Research Society (accepted).

Project 2: Circadian Entrainment, Sleep-Wake Regulation & Performance during Spaceflight

PI: Charles A. Czeisler, Ph.D, M.D.

Brigham and Women's Hospital/Harvard Medical School

Research Focus: Synchronization to Mars' day with two brief light pulses

Specific aims:

Test the hypotheses that:

1. Synchronization of the human circadian pacemaker to a sleep-wake and light-dark schedule with an imposed period ~4% longer than its intrinsic period will be disturbed.
2. This disturbed circadian synchronization will disrupt sleep, endocrine function, and impair waking alertness and performance.
3. Two relatively brief (45 minute) daily exposures to evening bright light (~10,000 lux) will establish a normal entrained circadian phase in subjects on such a schedule, resulting in improved sleep consolidation, undiminished growth hormone and cortisol secretion and enhanced daytime alertness and performance.

Research Progress 11/1/2003-10/31/2004:

We have designed a 65-day long between subjects randomized clinical trial to test the three hypotheses. Twelve subjects were randomly assigned to either Cell A, or B, or C. Subjects assigned to Cell A and Cell B were studied in a longer-than-24-hour sleep-wake schedule in approximately 25 and 100 lux in the angle of gaze during wakefulness, respectively. Subjects assigned to Cell C were studied in a longer-than-24-hour sleep-wake schedule in approximately 25 to 100 lux in the angle of gaze during wakefulness, but in addition, they were exposed to two brief 45 minute pulses of bright light (approximately 10,000 lux) in the evening, separated by 1-h of approximately 100 lux, as a countermeasure to entrain their pacemaker to the longer-than-24-hour day.

Since we completed the final experiments (12 subjects studied for 65 days each in total), data analyses have been actively computed. Data collected include: Core body temperature, blood samples (melatonin), Urine samples, Sleep and waking EEG recordings, Subjective sleep quality, Actigraphy, Light intensity, neurobehavioral performance and mood. Data analyses are currently in progress. The successful collection of the data will allow us to test hypotheses 1, 2, and 3 of the project. Plasma samples have been analyzed for melatonin, and period, circadian phase and phase angle of entrainment have been determined for all subjects throughout their 65-day study. Temperature data have been edited and also analyzed for estimation of intrinsic circadian period. Data analyses were completed for performance tests and mood questionnaires. Polysomnographic parameters of sleep EEG were analyzed (visual scoring of selected sleep episodes). Waking EEG analysis as a marker of alertness and brain activity during wakefulness is ongoing. Preliminary data have been presented in two major meetings (Congress of the Society for Research on Biological Rhythms, Canada in May 2004 and Congress of the European Sleep Research Society, Prague in October 2004). A manuscript is currently in preparation, its submission for publication is anticipated before the end of 2004.

We anticipated that subjects in cell A and B would fail to appropriately entrain their circadian pacemaker to the longer-than-24-hour day, resulting in sleep and endocrine disruption and impaired alertness and performance. We anticipated that subjects in Cell C, that is, scheduled to receive two relatively brief (45 minutes) daily exposures to evening bright light (approximately 10,000 lux) as a countermeasure to circadian misalignment, would establish a normal entrained circadian phase.

Our preliminary results indicate that subjects exposed to the two evening episodes of bright light demonstrate entrainment of their melatonin rhythms to the imposed sleep-wake cycle. They also reveal that lighting levels of approximately 25 lux (comparable to the intensities measured in the middeck for STS-95) were insufficient to maintain a normal phase angle of entrainment, and that approximately 100 lux (comparable to the intensities measured in the Spacehab for STS-95) was also insufficient to maintain a normal phase angle of entrainment in all subjects. Therefore, our preliminary results indicate that two relatively brief (45 minutes each) exposures to bright light in the evening are effective in maintaining entrainment of the circadian pacemaker to longer-than-24 h days, and would be effective as a countermeasure to circadian misalignment during space flight. Our data also suggest that ordinary room light (100-150 lux in the angle of gaze) might be sufficient to maintain entrainment to the 24.65-h solar day of Mars in a small number of astronauts, although at an abnormal phase angle that would be expected to induce physiological disturbances. Indeed, our data reveal that sleep (sleep structure, sleep efficiency, amount of slow wave sleep and of rapid eye movement sleep) is significantly altered in subjects who did not maintain a normal phase angle of entrainment. This study also shows that an abnormal phase angle is associated with disturbed alertness and performance during daytime. Further analysis reveals that both sleep structure and alertness/performance are closely related to the phase angle of entrainment (significant linear correlations). Hence, our study reveals that small alterations of phase angle of entrainment is expected to have major consequences on night- (sleep) and daytime (alertness and performance) functioning. Both the model and our preliminary data indicate that those astronauts who will have the greatest difficulty adapting to the 24.65 h Martian day will be those with an endogenous circadian pacemaker that has a period shorter than 24 h, which we estimate represents approximately 25 per cent of the population.

Optimal human performance during space flight requires astronauts to maintain synchrony between the circadian pacemaker which regulates the timing of sleep, endocrine function, alertness and performance and the timing of the imposed sleep-wake schedule. Operational demands of space flight necessitate that humans live on day lengths different than the 24-h solar day of Earth. Due to orbital mechanics, astronauts are commonly scheduled to the near equivalent of a shorter-than-24-hour day length in Earth orbit on space shuttle missions; moreover, they will be scheduled to the 24.65-h solar day of Mars on the planned exploration class mission to Mars. Through current support from the NSBRI and NASA, we have demonstrated that a scheduled dim light-dark rest-activity cycle, with a dim ambient light intensity similar to that used aboard the space shuttle middeck, is able to entrain most, but not all human subjects to a scheduled 24-hr day, whereas none of the human subjects scheduled to a 24.6-h day (the period of the axial rotation of Mars) were entrained to this weak synchronizer. Circadian phase misalignment to the 24.6-h day resulted in sleep disturbance (reduced sleep efficiency), endocrine disturbance (secretion of the sleep-promoting hormone melatonin during the waking day, reduced nocturnal growth hormone secretion and reduced cortisol levels), and impaired daytime alertness and neurobehavioral performance (reduced vigilance). The degree of circadian misalignment to the 24.6-h day was found to be strongly dependent upon the period of

each subject's circadian pacemaker, such that subjects with periods shorter than 24.0 hr demonstrated the greatest degree of circadian misalignment to the 24.6-h day. Due to concerns over high radiation exposure during the voyage to Mars and while on the planet's surface, NASA engineers have indicated that neither the spacecraft nor the Martian habitat may have windows. Our data suggest that most if not all astronauts would exhibit circadian misalignment if the space flight lighting conditions of approximately 10 lux on the windowless middeck of the space shuttle were present on the space craft while en route to Mars or on the Mars station during their approximately 540 day stay on Mars. The data collected during the past four years of support from the NSBRI demonstrate the need to develop effective and attainable countermeasures to prevent circadian misalignment during an exploration class mission to Mars. Our preliminary results suggest that exposures to bright light episodes of relatively short duration are effective in resetting the human circadian pacemaker and may provide an effective countermeasure to prevent circadian misalignment during exploration class space missions. This work has important implications for the treatment of circadian rhythm sleep disorders, such as advanced sleep phase syndrome and shift-work dysomnia, which are anticipated to have a high incidence and prevalence during exploration class space missions. Careful analysis of the efficacy of brief, intermittent bright light episodes in the treatment of these conditions has important ramifications for the practical application of bright light treatment during space flight, since repetitive uninterrupted exposure to bright light for many hours each day would not be feasible due to the cost of generating power and other operational demands of space missions. The results of our research could have a profound effect on the health, productivity and safety of astronauts during an exploration class mission to Mars. On earth, this work has important implications for the treatment of circadian rhythm sleep disorders, such as advanced sleep phase syndrome and shift-work dysomnia, which are anticipated to have a high incidence and prevalence during exploration class space missions.

We have designed a 65-day long between subjects randomized clinical trial to test the three hypotheses. Twelve subjects were randomly assigned to either Cell A, or B, or C. Subjects assigned to Cell A and Cell B were studied in a longer-than-24-hour sleep-wake schedule in approximately 25 and 100 lux in the angle of gaze during wakefulness, respectively. Subjects assigned to Cell C were studied in a longer-than-24-hour sleep-wake schedule in approximately 25 to 100 lux in the angle of gaze during wakefulness, but in addition, they were exposed to two brief 45 minute pulses of bright light (approximately 10,000 lux) in the evening, separated by 1-h of approximately 100 lux, as a countermeasure to entrain their pacemaker to the longer-than-24-hour day.

Since we completed the final experiments (12 subjects studied for 65 days each in total), data analyses have been actively computed. Data collected include: Core body temperature, blood samples (melatonin), Urine samples, Sleep and waking EEG recordings, Subjective sleep quality, Actigraphy, Light intensity, neurobehavioral performance and mood. Data analyses are currently in progress. The successful collection of the data will allow us to test hypotheses 1, 2, and 3 of the project. Plasma samples have been analyzed for melatonin, and period, circadian phase and phase angle of entrainment have been determined for all subjects throughout their 65-day study. Temperature data have been edited and also analyzed for estimation of intrinsic circadian period. Data analyses were completed for performance tests and mood questionnaires. Polysomnographic parameters of sleep EEG were analyzed (visual scoring of selected sleep episodes). Waking EEG analysis as a marker of alertness and brain activity during wakefulness

is ongoing. Preliminary data have been presented in two major meetings (Congress of the Society for Research on Biological Rhythms, Canada in May 2004 and Congress of the European Sleep Research Society, Prague in October 2004). A manuscript is currently in writing, its submission for publication is anticipated for the end of this year.

We anticipated that subjects in cell A and B would fail to appropriately entrain their circadian pacemaker to the longer-than-24-hour day, resulting in sleep and endocrine disruption and impaired alertness and performance. We anticipated that subjects in Cell C, that is, scheduled to receive two relatively brief (45 minutes) daily exposures to evening bright light (approximately 10,000 lux) as a countermeasure to circadian misalignment, would establish a normal entrained circadian phase.

Our preliminary results indicate that subjects exposed to the two evening episodes of bright light demonstrate entrainment of their melatonin rhythms to the imposed sleep-wake cycle. They also reveal that lighting levels of approximately 25 lux (comparable to the intensities measured in the middeck for STS-95) were insufficient to maintain a normal phase angle of entrainment, and that approximately 100 lux (comparable to the intensities measured in the Spacehab for STS-95) was also insufficient to maintain a normal phase angle of entrainment in all subjects. Therefore, our preliminary results indicate that two relatively brief (45 minutes each) exposures to bright light in the evening are effective in maintaining entrainment of the circadian pacemaker to longer-than-24 h days, and would be effective as a countermeasure to circadian misalignment during space flight. Our data also suggest that ordinary room light (100-150 lux in the angle of gaze) might be sufficient to maintain entrainment to the 24.65-h solar day of Mars in a small number of astronauts, although at an abnormal phase angle that would be expected to induce physiological disturbances. Indeed, our data reveal that sleep (sleep structure, sleep efficiency, amount of slow wave sleep and of rapid eye movement sleep) is significantly altered in subjects who did not maintain a normal phase angle of entrainment. This study also shows that an abnormal phase angle is associated with disturbed alertness and performance during daytime. Further analysis reveals that both sleep structure and alertness/performance are closely related to the phase angle of entrainment (significant linear correlations). Hence, our study reveals that small alterations of phase angle of entrainment is expected to have major consequences on night- (sleep) and daytime (alertness and performance) functioning. Both the model and our preliminary data indicate that those astronauts who will have the greatest difficulty adapting to the 24.65 h Martian day will be those with an endogenous circadian pacemaker that has a period shorter than 24 h, which we estimate represents approximately 25 per cent of the population.

Optimal human performance during space flight requires astronauts to maintain synchrony between the circadian pacemaker which regulates the timing of sleep, endocrine function, alertness and performance and the timing of the imposed sleep-wake schedule. Operational demands of space flight necessitate that humans live on day lengths different than the 24-h solar day of Earth. Due to orbital mechanics, astronauts are commonly scheduled to the near equivalent of a shorter-than-24-hour day length in Earth orbit on space shuttle missions; moreover, they will be scheduled to the 24.65-h solar day of Mars on the planned exploration class mission to Mars. Through current support from the NSBRI and NASA, we have demonstrated that a scheduled dim light-dark rest-activity cycle, with a dim ambient light intensity similar to that used aboard the space shuttle middeck, is able to entrain most, but not all human subjects to a scheduled 24-hr day, whereas none of the human subjects scheduled to a 24.6-h day (the period of the axial rotation of Mars) were entrained to this weak synchronizer. Circadian phase misalignment to the 24.6-h day resulted in sleep disturbance (reduced sleep

efficiency), endocrine disturbance (secretion of the sleep-promoting hormone melatonin during the waking day, reduced nocturnal growth hormone secretion and reduced cortisol levels), and impaired daytime alertness and neurobehavioral performance (reduced vigilance). The degree of circadian misalignment to the 24.6-h day was found to be strongly dependent upon the period of each subject's circadian pacemaker, such that subjects with periods shorter than 24.0 hr demonstrated the greatest degree of circadian misalignment to the 24.6-h day. Due to concerns over high radiation exposure during the voyage to Mars and while on the planet's surface, NASA engineers have indicated that neither the spacecraft nor the Martian habitat may have windows. Our data suggest that most if not all astronauts would exhibit circadian misalignment if the space flight lighting conditions of approximately 10 lux on the windowless middeck of the space shuttle were present on the space craft while en route to Mars or on the Mars station during their approximately 540 day stay on Mars. The data collected during the past four years of support from the NSBRI demonstrate the need to develop effective and attainable countermeasures to prevent circadian misalignment during an exploration class mission to Mars.

III. KEY FINDINGS AND IMPLICATIONS FOR FUTURE RESEARCH

Our preliminary results suggest that exposures to bright light episodes of relatively short duration are effective in resetting the human circadian pacemaker and may provide an effective countermeasure to prevent circadian misalignment during exploration class space missions. This work has important implications for the treatment of circadian rhythm sleep disorders, such as advanced sleep phase syndrome and shift-work dyssomnia, which are anticipated to have a high incidence and prevalence during exploration class space missions. Careful analysis of the efficacy of brief, intermittent bright light episodes in the treatment of these conditions has important ramifications for the practical application of bright light treatment during space flight, since repetitive uninterrupted exposure to bright light for many hours each day would not be feasible due to the cost of generating power and other operational demands of space missions. The results of our research could have a profound effect on the health, productivity and safety of astronauts during an exploration class mission to Mars. On earth, this work has important implications for the treatment of circadian rhythm sleep disorders, such as advanced sleep phase syndrome and shift-work dyssomnia, which are anticipated to have a high incidence and prevalence during exploration class space missions. Future direction for the coming four years include evaluation of the efficacy of short-wavelength (blue) enhanced ambient light to prevent circadian misalignment during.....

Project 3: Countermeasures to Neurobehavioral Deficits From Partial Sleep Loss

PI: David F. Dinges, Ph.D.
University of Pennsylvania

Research Focus: The use of naps to ameliorate the affects of chronic sleep restriction

Specific aims:

1. Establish Response Surface Map to determine how to best use anchor and nap sleep periods to promote neurobehavioral performance and alertness at an adverse circadian phase for waking;
2. Identify the optimal diurnal anchor sleep and nocturnal nap schedule to maintain neurobehavioral function when work is initiated with abrupt circadian displacement;
3. Determine how diurnal anchor sleep times and nocturnal nap sleep affect sleep physiology and circadian adjustment across a chronic schedule of simulated night operations.

Research Progress 11/1/2003-10/31/2004:

A. Specific Aims

The overarching goal of this project is to develop sleep schedule countermeasures to ensure optimal neurocognitive performance capability in astronauts during prolonged space flight. The primary aim is to determine the sleep dose-response effects of an acute change in sleep duration that occurs between two periods of chronic sleep restriction, on neurocognitive performance functions, subjective states, and waking and sleep physiology. The project is relevant to the NSBRI Human Performance Factors, Sleep and Chronobiology research area and strategic plan risk-based goal 2 and non-risk-based goals 9 and 10. It addresses questions 6.50, 6.06, 6.10, and 6.15 in Critical Path Roadmap areas 19, 20 and 21 under Human Behavior and Performance (see Form F). The proposed research is at countermeasure readiness level 5.

The optimal performance of astronauts during extended-duration space flight depends heavily on achieving recovery through adequate sleep. There is now extensive evidence that astronaut sleep in space averages 4 to 6.5 hours per day, and when critical operations (e.g., nighttime docking) are scheduled, very little sleep may be obtained during a day prior to the critical event. Ground-based experiments on healthy adults by our laboratory and others have demonstrated that limiting daily sleep duration to less than 7 hours leads to cumulative deficits in neurocognitive performance and alertness. Within 1-2 weeks of sleep restriction at levels experienced by astronauts, performance deficits were serious; impairments on tasks requiring sustained attention, working memory and cognitive throughput reached levels equivalent to those found after 1-2 nights of total sleep loss.

The focus of our research has been on finding countermeasures to prevent cumulative performance deficits from occurring in space flight, given that chronic sleep restriction is unavoidable due to mission demands. To date our research for NSBRI has involved studies of n=140 healthy young adults (~2,000 laboratory days) to determine if any daily combination of reduced sleep and a brief nap could prevent cumulative performance deficits. These response surface modeling experiments have shown that a daily nap can mitigate some performance degradation during chronic partial sleep loss, but total sleep time per 24h remains the prime determinant of cognitive performance. Whenever daily total sleep dosage was <7h—irrespective of split sleep schedules or the circadian phase at which sleep was obtained—performance deficits accumulated across days. Since a split schedule involving anchor sleep and a nap was found to be only a partial countermeasure to the cumulative effects of chronic sleep restriction, we now seek to determine if a single recovery sleep episode midway in a chronic period of sleep restriction can provide another sleep countermeasure option.

The proposed experiment will determine the countermeasure benefits for performance (during critical operations and subsequent days of sleep restriction) from an acute increase in sleep duration (i.e., single night of recovery sleep). In addition, generating sleep dose-response functions will provide critically needed information on the adverse performance consequences of an acute reduction in sleep duration below the chronic sleep-restriction level, which can occur in space flight prior to a day of critical operations. The project has the following four specific aims:

1. We will **establish sleep dose-response curves (S-DRCs) for the immediate and delayed impact on neurobehavioral functions, of an acute (1 night) change in sleep duration midway in a period of chronic sleep restriction.** We will test the following two hypotheses.

Hypothesis 1a: As time in bed (TIB) for sleep increases on the acute intervention night, following chronic sleep restriction (days pa1 to pa6 in Figure D1), performance on the next day of simulated critical operations (day i-drc) will improve in a sleep duration dose-response manner. Hypothesis 1b: As TIB for sleep increases on the acute intervention night (i-drc), performance on the subsequent 6 days of chronic sleep restriction (days pb1 to pb6) will improve in a sleep duration dose-response manner. By generating S-DRCs for an acute sleep intervention (i-drc), we will also be able to identify the breakpoint in DRC functions, which is the point at which further increases in sleep duration will yield both acute (hypothesis 1a) and sustained (hypothesis 1b) performance benefits. To accomplish this aim, a range of relevant neurobehavioral functions (e.g., cognitive performance on WinSCAT, PVT, NTB, MiniCOG, etc.; subjective sleepiness; mood states) and physiological responses (e.g., waking EEG, slow eyelid closures during simulated driving) will be evaluated throughout a 17-day laboratory protocol in N=80 healthy adult females (n=40) and males (n=40) aged 21-45y. Subjects will be randomized to one of seven different acute sleep duration interventions (i-drc = 0h, 2h, 4h, 6h, 8h, 10h, or 12h TIB; n=10 in each condition) midway in a schedule of chronic restriction of sleep to 4h TIB/night. An additional control condition (n=10) will involve 10h TIB for sleep on all 17 nights of the protocol. To establish the shape of the resulting S-DRCs, the best-fitting function will be selected from a series of generically formulated mathematical curves on the basis of a statistical information criterion. Analyses will account for gender differences, age effects, individual differences in performance ability, morningness-eveningness, and the influence of habitual sleep durations.

2. We will **determine if performance recovery is complete after 2 nights of extended sleep,** following chronic sleep restriction. In addition to the impact of a single night intervention (specific aim 1), we seek to resolve whether complete neurobehavioral recovery from prolonged chronic sleep restriction is possible within 2 nights. Using the same experimental design, we will test the following hypotheses. Hypothesis 2a: In the seven chronic sleep restriction conditions, performance functions will continue to show significant improvement on the second day (r2) of 10h TIB recovery sleep relative to the first day (r1). Hypothesis 2b: Relative to the 10h TIB control condition, the chronic sleep restriction conditions will result in poorer performance on final recovery day 1 (r1), but not final recovery day 2 (r2). We selected 2 nights at 10h TIB, to ensure subjects receive more than 8h physiological sleep, but within TIB limits that would be practical in space flight.

3. We will **investigate the relationship between sleep physiology and performance responses.** In this specific aim we will evaluate dynamic changes in sleep physiology (across

days) using polysomnographic variables and sleep EEG nonREM delta power (SWA; the putative marker of sleep homeostasis), to gain insights into how sleep physiology relates to waking neurobehavioral functions. We will test Hypothesis 3: Total sleep time during the acute intervention night (i-drc) will be a greater predictor of both acute and sustained performance recovery (specific aim 1) than will either specific sleep stages or SWA. In additional exploratory analyses we will evaluate sleep physiological responses for all conditions during both 6-day periods of chronic sleep restriction and final 2 recovery-sleep days.

4. We will **investigate the effects of chronic sleep restriction, acute sleep intervention, and recovery sleep on cardiovascular indices**. This specific aim is exploratory and hypothesis generating. It is based on previous work we have conducted demonstrating alterations in cardiovascular and autonomic markers (i.e., resting heart rate [HR]) and inflammatory markers (increased plasma levels of C-reactive protein [CRP]) during periods of sleep loss. We seek to determine the effects that chronic sleep restriction and variable durations of acute sleep intervention have on these markers.

Executive Summary:

This project is concerned with identifying methods to prevent neurobehavioral and physical deterioration due to inadequate sleep and sleep placed at different times across the 24-hour day in astronauts during long-duration manned space flight. The performance capability of astronauts during extended-duration space flight depends heavily on achieving recovery through adequate sleep. Even with appropriate circadian alignment, sleep loss can erode fundamental elements of human performance capability including vigilance, cognitive speed and accuracy, working memory, reaction time, and physiological alertness. When attempting to sleep and perform at an adverse circadian phase, the magnitude and time course of sleep loss and consequent deficits in neurobehavioral functioning are significantly affected. Adequate sleep is essential during manned space flight not only to ensure high levels of safe and effective human performance, but also as a basic regulatory biology critical to healthy human functioning.

There is extensive objective evidence that astronaut sleep is restricted in space flight to averages between 4 hr and 6.5 hr/day. Chronic sleep restriction during manned space flight can occur in response to endogenous disturbances of sleep (motion sickness, stress, circadian rhythms), environmental disruptions of sleep (noise, temperature, light), and curtailment of sleep due to the work demands and other activities that accompany extended space flight operations. The mechanism through which this risk emerges is the development of cumulative homeostatic pressure for sleep across consecutive days of inadequate sleep. Research has shown that the physiological sleepiness and performance deficits can progressively worsen (i.e., accumulate) over consecutive days of sleep restriction, and that sleep limited to levels commonly experienced by astronauts (i.e., 4 - 6hr per night) for as little as 1 week, can result in increased lapses of attention, degradation of response times, deficits in complex problem solving, reduced learning, mood disturbance, disruption of essential neuroendocrine, metabolic, and neuroimmune responses, and in some vulnerable persons, the emergence of uncontrolled sleep attacks.

The prevention of cumulative performance deficits and neuroendocrine disruption from sleep restriction during extended duration space flight involves finding the most effective ways to obtain sleep in order to maintain the high-level cognitive and physical performance functions required for manned space flight. There is currently a critical deficiency in knowledge of the effects of how

variations in sleep duration and timing relate to the most efficient return of performance per unit time invested in sleep during long-duration missions, and how the nature of sleep physiology (i.e., sleep stages, sleep electroencephalographic [EEG] power spectral analyses) changes as a function of sleep restriction, the timing of sleep, and performance degradation. The primary aim of this project is to meet these critical deficiencies through utilization of a response surface experimental paradigm. Through testing in a dose-response manner, varying combinations of sleep duration and timing, this project will help establish how to most effectively limit the cumulative adverse effects of chronic sleep restriction in space operations on human performance and physiology.

Although there is evidence that the less sleep obtained, the greater the waking deficits, experiments have found that for acute periods supplementing a reduced anchor sleep period with a nap has the potential to enhance performance, due to the exponential recovery of neurobehavioral functions relative to sleep duration. During the past 5 years we have been using a response surface experimental approach to systematically determine the chronic (10-day) effects of 18 sleep schedule conditions. There are two experiments in this project. The first experiment involved restricted nocturnal anchor sleep alone and in combination with varying durations of restricted daytime naps on performance, mood, sleep, circadian physiology and hormones. The resulting preliminary response surface maps (RSMs) derived from this dose-response experiment indicate that total sleep time per 24hr is a prime determinant of cumulative neurobehavioral deficits, and that combining a restricted nocturnal anchor sleep with a midday nap can attenuate cumulative deterioration in performance. In order to complete our understanding of how to optimize performance in the face of restricted sleep in space flight, in the second experiment we have reversed the circadian placement of these 18 anchor sleep + nap sleep conditions (i.e., daytime anchor sleep alone and in combination with varying

Project Number: HPF00204 Principal Investigator: David F. Dinges, Ph.D. Page 2
durations of restricted nocturnal naps.

To develop the response surface models, both experiments will require $n=90$ (total $N=180$) healthy men and women to undergo a 14-day ground-based laboratory protocol involving random assignment to one of 18 sleep-ration cells. The 18 sleep ration cells utilized in experiment 1 (nocturnal anchor sleep) will be repeated in experiment 2 (diurnal anchor sleep) for a total of 36 sleep ration cells. The sleep-ration assignments involve 4 anchor sleep durations (4.2, 5.2, 6.2, 8.2 hr) and 6 nap sleep durations (0.4, 0.8, 1.2, 1.6, 2.0, 2.4 hr) crossed to yield a total of 4 anchor-sleep-only conditions, and 14 anchor + nap sleep conditions, and spanning a dynamic range of cumulative sleep debts (i.e., from 0 to 40 hr in a 10-day period). Subjects undergo a wide range of quasi-continuous neurobehavioral performance tests and continuous physiological monitoring of waking EEG, sleep PSG, behavioral motility, and body temperature, while living in the laboratory for 14 consecutive days. The laboratory environment is designed to simulate the low light, tight quarters, and lack of social contact with the outside world that will characterize long-duration space flight.

CRITICAL PATH ROADMAP (<http://criticalpath.jsc.nasa.gov/>)

CPR Area Human performance failure due to sleep and circadian problems (19)

CPR Version May 2003

CPR Risk(s) Addressed:

30 Human Performance Failure Due to Sleep Loss and Circadian Rhythm Problems

Critical Questions Addressed:

None

Specific Aims:

The focus of our research is on determining how variations in sleep duration and its circadian placement relate to the most efficient return of performance and alertness per unit time invested in sleep, in order to establish whether there is a way to optimize performance in the face of restricted sleep during space flight. The project has the following three specific aims.

1. Establish response surface maps (RSMs) to determine how to best use anchor and nap sleeps to promote neurobehavioral performance and alertness at an adverse circadian phase for waking.
2. Identify the optimal diurnal anchor sleep and nocturnal nap schedule to maintain neurobehavioral function when work is initiated with abrupt circadian displacement.
3. Determine how diurnal anchor sleep times and nocturnal nap sleep affect sleep physiology and circadian adjustment across a chronic schedule of simulated night operations.

Main Findings in Reporting Year and Contributions to Answering Critical Questions:

Preliminary analyses on the neurobehavioral data obtained from experiment 2 have been completed. Descriptive analyses of the data obtained thus far supports the findings from experiment 1. That is, Project Number: HPF00204 Principal Investigator: David F. Dinges, Ph.D. Page 3 achieving physiological sleep on split-sleep schedules was possible, and naps helped attenuate some cumulative impairments, but TST per 24h (by PSG) remained the prime determinate of behavioral capability over time. Moreover, naps taken at the nocturnal circadian phase, resulted in severe sleep inertia upon awakening, which had to dissipate before nap benefits on performance were seen.

Preliminary analysis of the endocrine data demonstrate significant effects of both sleep restriction and circadian disruption on melatonin and cortisol profiles. In the 8.2h anchor sleep condition, with no nap, a significant delay in the timing of the melatonin profile was evident on the last day of restriction compared to the baseline day. In addition, a decrease in the amplitude of the melatonin curve was evident. When sleep was chronically restricted to 4.2h per diurnal anchor sleep period, with and without naps, a greater degree of variability in the melatonin profile was evident. Some subjects demonstrated a phase delay in the timing of the melatonin rhythm, while other subjects experienced an advance or no change in the timing of melatonin secretion.

Changes in the cortisol profile on the final day of the restriction period compared to the baseline day were also evident. Timing of the morning peak in cortisol levels was altered on the final restriction day, and a decrease in the amplitude of the cortisol profile was also observed in subjects allowed 8.2h diurnal sleep per day and 4.2h diurnal sleep, with and without naps.

Unique Claims of Study:

This experiment is the first ground-based study to utilize the slopes of cumulative neurobehavioral deficits and physiological changes across days of chronic sleep restriction, with sleep placed at

different circadian times, to determine the extent to which the duration and timing of sleep per 24 hours (in the range commonly experienced by astronauts in flight) and the use of combined anchor + nap sleep opportunities each 24 hours, can prevent or attenuate development of cumulative fatigue and performance deficits. The response surface experimental paradigm affords a high return of information regarding the optimal way to utilize sleep in operations that inherently limit time for sleep, as in the space flight environment. The results of our research will contribute to the optimization of performance, productivity, safety and health during extended missions, by providing astronauts with the most efficient sleep-wake schedules. The results will also address critical path questions by (1) determining both the acute and long-term neurobehavioral and physiological effects of exposure to restricted sleep durations placed at different times of the 24-hour day, in the range commonly experienced by astronauts in space flight; (2) establishing whether sleep-wake schedule countermeasures involving varying combinations of restricted anchor sleep and nap sleep, at different circadian times, can effectively mitigate the performance risks posed to astronauts by chronically restricted sleep in space flight; (3) providing estimates of any potentially adverse effects of optimal sleep schedule countermeasures on hormonal profiles, sleep inertia and related physiological functions; and (4) providing performance technologies and needed data for the development of a biomathematical model of human performance capability relative to sleep schedules and circadian physiology. These techniques will ultimately aid astronauts in the self-management of sleep and alertness during long-duration space flight.

This project specifically addresses the following questions in the Human Performance Factors, Sleep and Chronobiology research area:

1. Which countermeasure or combination of behavioral and physiological countermeasures will optimally mitigate specific performance problems associated with sleep loss and circadian disturbances during a Mars mission? (Bioastronautics Critical Path Roadmap question 6.06)
2. What are the acute and long term effects of exposure to the space environment on biological rhythmicity on sleep architecture, quality and quantity, and their relationship to performance capability? (Bioastronautics Critical Path Roadmap question 6.05)
3. What workload schedule(s) per workday will best enhance crew performance and mitigate adverse effects of the space environment? (Bioastronautics Critical Path Roadmap question 6.10)

Project 4: Novel Photoreceptor Mechanisms Regulating Circadian rhythms, Sleep, Body Temperature and Heart Rate.

PI: *Russell Foster, Ph.D.*
Imperial College, London, UK

Research Focus: The impact of non-rod, non-cone ocular photoreception on physiology and behavior.

Specific aims:

1. To determine the role of novel photoreceptors in the regulation of general physiology and behavior. The experiments in this section will determine the extent to which body temperature, heart rate (ECG) and EEG are modulated by non-rod, non-cone ocular photoreceptors using a unique mouse model (*rd/rd cl*), which lacks all rod and cone photoreceptors.

2. To define the relationship between novel ocular photoreceptors, light, sleep state and levels of *c-fos* expression in the ventrolateral preoptic nucleus (VLPO) of the brain. The VLPO integrates light information to modulate sleep state. We will establish whether the light information that reaches this brain structure is primarily from the rods, cones, novel receptors, or a combination thereof.

3) To characterize the molecular mechanisms of non-rod, non-cone ocular photoreception. Three broad strategies will be undertaken to look for these molecules based upon bioinformatics, proteomics, and micro array technology. Our aim will be to identify a set of genes that: a) share sequence similarity with proteins known to be involved in photoreceptor/sensory cell function; b) are expressed in light sensitive cells of the inner retina; c) undergo post-translational modification and/or changes in expression following light exposure. We will subsequently confirm that these genes are expressed in candidate photoreceptive cells using *in situ* hybridization, single cell PCR from photosensitive retinal neurons, and the generation of suitable knockouts where appropriate.

Research Progress 11/1/2003-10/31/2004:

Sub-Project Title: The Role and Characterization of Novel Photoreceptor Mechanisms Regulating Circadian rhythms, Sleep, Body Temperature and Heart Rate: Implications for Creating Artificial Light Environments in Space.

Below we summarize the progress made on the two questions formulated in our original application.

Question 1: What is the role of novel photoreceptors in the regulation of general physiology and behavior?

1a) Modulation of sleep by non-rod non-cone photoreceptors in rodless coneless (*rd/rd cl*) mice. Sleep and wakefulness are not only associated with changes in neural activity but also with differences in gene expression, including the immediate early gene *c-fos*. During wakefulness there is a selective induction of *c-fos* mRNA and protein in brain regions that contain wake-active neurones (e.g. cerebral cortex and tuberomammillary nucleus). By contrast, during sleep, *c-fos* expression is very low in wake active brain structures but high in regions that modulate sleep, such as the hypothalamic ventrolateral preoptic nucleus (VLPO). In rodents, *c-fos* expression in the VLPO is associated with elevated light and sleep induction. To determine if there is a direct relationship between light, non-rod, non-cone photoreceptors, Fos induction and sleep we used Fos expression as a marker for neuronal activation in the VLPO of rodless coneless (*rd/rd cl*) mice. We delivered a monochromatic light pulse four hours after the onset of darkness and performed Fos immunohistochemistry followed by densitometry image analysis. Additionally, we performed anterograde tract tracing with the carbocyanine fluorescent lipophilic tracer DiI to identify direct projections from the retina to the VLPO of *rd/rd cl* mice. The results show a four fold increase in Fos in the VLPO after light exposure compared to dark control animals (Figure 1).

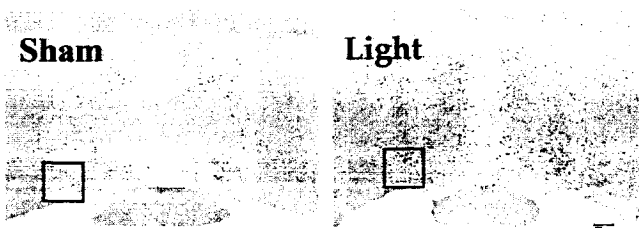


Figure 1. Fos induction in the VLPO (box) of an *rd/rd cl* mouse. There is a four fold

greater level of Fos expression in the light treated compared to the sham control.

We also show the presence of direct retinal projections to the VLPO in these mice. We suggest that light directly influences the activation of sleep-promoting neurones in the VLPO of *rd/rd cl* mice. ***Collectively our findings provide the first physiological evidence that non-rod non-cone photoreceptors modulate sleep in mice.***

1b) The photic regulation of the cardiovascular system. Cardiovascular performance is modified through central feed-forward mechanisms that are components of more complex behavioural or physiological responses, such as cardiovascular changes associated with exercise and threat or alerting stimuli. However, light responses in cardiovascular parameters have only been poorly characterised. Therefore, the photoreceptive systems regulating HR were investigated simultaneously in both wildtype and *rd/rd cl* mice, by implanted radio-telemetry bio-potential recording. Equipment has been sourced and customised to allow simultaneous recording of radio-telemetry and CCTV, in conjunction with 'home cage' light pulsing within environment control cabinets. Implanted radio-telemetry transmitters provide a capacity to make long-term on-line measurements in mice that have uninhibited movement and are free of pharmacological artefacts. CCTV recording allows independent assessment of activity so that the basis of responses can be more confidently assigned to the given stimuli. A system of fibre optics and light sources allows light conditions within cabinets to be defined. Equipment was configured to record electroencephalogram (ECG), core body temperature (CBT) and activity at 90-second intervals. Activity was independently described by scoring of CCTV records to a strict activity rating scale. Baselines and circadian rhythms of ECG, CBT and activity were defined in animals entrained to a 12:12 L/D cycle and free-running in D/D. 10-minute white light pulses were applied remotely to animals in 'home cages' to describe light responses in ECG, CBT and activity.

The circadian rhythm in HR consistently preceded CBT and activity in both genotypes suggest a pre-emptive change in arousal state preparatory to sustained changes in behaviour, consistent with the adaptive advantage assigned to endogenous circadian rhythms. Interestingly, changes in the circadian rhythm of CBT appear to be pre-emptive of changes in activity and therefore muscular activity related heat production. This might identify the action of a direct regulatory mechanism. The wildtype exhibits a rapid response in HR to light (Figure 2a). The increase in HR has no identifiable lag, reaches a maximum within ~3 minutes, is generally sustained for the duration of the brief light pulse and is often followed by an elevation of CBT and a bout of activity. By contrast *rd/rd cl* have no identifiable response in HR to light (Figure 2b). ***These preliminary data suggest that the acute regulation of heart rate by environmental irradiance arise from inputs from the classical rod and cone photoreceptor pathways.***

Figure 2: Wildtype (A) and rdrd cl (B) responses to a $407 \mu W cm^2 s^{-1}$ white light pulse (red bar).

A

<http://www.nsbri.org/intranet/events.epl>

B

Question 2: What are the molecular mechanisms of non-rod, non-cone ocular photoreceptors?

2a) Bioinformatics. Our recent melanopsin knockout studies are unable to establish the biochemical function of this protein and there is still uncertainty over whether it acts as a sensory photopigment or in an accessory role within inner retinal photoreceptors. We have therefore employed a bioinformatics approach to identify additional novel opsin genes in the mammalian genome. Two search strategies were undertaken using either opsin nucleotide or protein sequences. These sequences included the classic opsins such as the rod and cone opsins, as well as novel opsins such as pinopsin (P-opsin), vertebrate ancient (VA) opsin and melanopsin. In the initial search the BLASTN algorithm (available from www.ncbi.nlm.nih.gov/blast) was used to screen public databases such as GenBank with a representative selection of opsin nucleotide sequences. We were unable to identify any novel opsin sequences with this approach. However, a second screen of the databases that utilised the TBLASTN algorithm with the protein sequences of members of the various opsin families was successful. Sequences that showed a match at the crucial lysine residue that corresponds to the site of retinal attachment in all known opsin proteins were further analysed. *Two human EST sequences were identified, obtained as IMAGE clones from the HGMP Resource Centre and sequenced. Subsequent sequence analysis showed that we have identified a novel opsin (termed OPN5 or neuropsin), which is localised on human chromosome 6p12. RT-PCR analysis has demonstrated expression of this novel opsin in human retina.* NOP shows highest similarity to invertebrate-like opsins (mammalian melanopsin) at around 30 % identity. A full cDNA sequence of human neuropsin and the mouse orthologue has been determined and RT-PCR analysis on a panel of mouse tissues has shown that OPN5 is expressed in the mouse eye (Tarttelin *et al.*, 2003). We will complete a more detailed examination of its sites of expression in mice using *in situ* hybridisation histochemistry. We have also generated an antibody to the mouse OPN5 protein, which is being employed in Western blot and Immunocytochemical analyses. Finally, we have identified orthologues of OPN5 in zebrafish and *Xenopus* which are also expressed only in neural tissue.

2b) Microarray Project. To investigate changes in gene expression in the eye following acute light exposure, we have undertaken a microarray project using *rd/rd cl* mice. By using the *rd/rd cl* model, lacking classical photoreceptors, we can investigate the pathways involved in non-image forming responses to light. Our progress to date is summarised here.

Rodless+ coneless *rd/rd cl* mice were housed under a 12:12 LD cycle for 3 weeks before a 15 minute bright white light pulse was administered (~2000 lux). Animals were killed by cervical dislocation and eyes were collected and snap frozen on dry ice. All procedures were conducted in darkness using infrared viewers. Tissue was collected at 30 mins, 60 mins and 120 mins after the start of light exposure. Time matched sham controls were included at each time point. For each time point *n*=6 for pulsed animals, with *n*=6 matched controls (total 36 animals). RNA was then extracted from whole eyes using Trizol, and quantity and quality was assessed by spectrophotometry and use of an Agilent Bioanalyser (using the eukaryotic RNA nano chip). In all cases between 10 and 20 ug of total RNA was obtained, all of acceptable quality for array hybridisation. This RNA is currently ready for *in-vitro* transcription and labelling, ready for genechip analysis. Genome-wide expression of over 39,000 transcripts will shortly be conducted using the Affymetrix murine 430 2.0 whole genome arrays, within the following month.

Publications:

Tarttelin, E.E., Bellingham, J., Hankins, M.W., Foster, R.G. & Lucas, R.J. (2003) Neuropsin (Opn5): a novel opsin identified in mammalian neural tissue. *FEBS Lett*, 554, 410-416.

Project 5: Primate Circadian Rhythms in the Martian Environment

PI: Charles A. Fuller, Ph.D.
University of California, Davis

Research Focus: Bright light pulses, Period vs. Gravity

Specific aims:

Test the hypotheses that:

1. Rhesus macaques will not entrain to the Martian solar day when exposed to ambient light available on Mars, resulting in performance decrements and sleep and circadian dysfunction;
2. Some, but not all rhesus macaques will be able to entrain to a Martian solar day under a lighting environment proposed for the Martian Habitat;
3. The rhesus circadian period will change as a direct function of G level in hypergravity;
4. Daily evening pulses of bright light will synchronize all rhesus monkeys to the proposed Habitat environment

Research Progress 11/1/2003-10/31/2004:

1. Effect of Gravity on the Circadian Period of Rhesus Monkeys

During this period studies of female rhesus (n=5) in 1, 1.5 and 2.0G were completed to complement those completed with male rhesus during the preceding year. Circadian periods were determined in 1 and 2G based on brain temperature rhythms. Although there was evidence for an effect of G on some circadian responses, a significant effect of G on circadian period was not demonstrated. However, periods tended to be slightly longer in 2G, consistent with findings from another primate species. The females were tested in constant dim light (LL) as well as in forced desynchrony (FD) and although periods were slightly longer in 2G in both conditions we were not able to show significant differences. The principal effect of altered G observed in these studies was reduced sensitivity of the animals to the ambient LD cycle as shown by reduced masking of the brain temperature rhythm. This is consistent with prior demonstrations of decreased light sensitivity in other species in hypergravity, and with our preliminary observation that entrainment of several of the male rhesus was impaired in 2G. Reduced LD masking was evident in both a baseline 24-h day (LD 16:8) and in forced desynchrony for both males and females, and overall for LD, FD and LL for the females. Evaluation of sleep and performance data is ongoing and manuscripts are being prepared as data evaluation is completed.

2. Acclimation to Martian Day Length and Altered Lighting Environments

Studies were completed comparing entrainment to a martian-length day (24.67 h) with a terrestrial 24-h day in male rhesus monkeys. Entrainment was compared between habitat and red-enriched lighting spectra. These studies were complementary to prior studies involving female rhesus monkeys. For both genders, circadian periods were obtained in forced desynchrony allowing us to compare the animals' endogenous periods with the martian daylength. Both genders were able to entrain well to the martian day and exhibited normal feeding and behavior. Circadian periods for the rhesus were on average slightly longer than in

humans, thus possibly in part accounting for the ease of entrainment since smaller daily phase-shifts would have been required to maintain entrainment to the martian day. Evaluation of sleep and performance data is ongoing and manuscripts are being prepared as data evaluation is completed.

Significance of Findings

Although our findings support the ability of the circadian timing system (CTS) to adapt to altered gravity environments, they also support concerns that this adaptation presents difficulties. Our findings support reduced responsiveness of the CTS to ambient light cycles in an altered G environment, something previously seen in nocturnal rodents. Thus we are concerned that light countermeasures developed in a terrestrial environment may not have the same efficacy in altered G. We intend to pursue this issue in future studies. Although the current findings relating to the effect of altered G on intrinsic period of the CTS were not conclusive, we believe that taken in combination with prior findings there is a good case to be made for alteration of basic clock properties in altered gravity. We intend to pursue this area of concern as well in future studies. The interaction of direct G-effects on the CTS and altered sensitivity to light input to is still largely uncharacterized and is an area with major implications for countermeasure evolution. With artificial gravity (AG) as a likely countermeasure for a number of physiological changes seen during long-duration space flight it is imperative that the G-sensitivity of the CTS and its light-responsiveness to altered G be better understood. Among potential concerns is that the timing of light and AG countermeasures be coordinated in such a way as to ensure that one countermeasure does not compromise the benefits of the other.

Project 6: Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure

*PI: Elizabeth B. Klerman, M.D., Ph.D.
Brigham and Women's Hospital/Harvard Medical School*

Research Focus: Use of light and performance mathematical models in schedule design

Summary:

- 1) We completed work on grant HPF00203. "Human Performance Factors, Sleep and Chronobiology Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure"
- 2) We began work on grant "Mathematical Models of Circadian/Performance Countermeasures 2004"

Project 1:

Report for grant "Human Performance Factors, Sleep and Chronobiology Mathematical Model for Scheduled Light Exposure: Circadian/Performance Countermeasure"
HPF00203

Ended 3/31/04. This grant was completed in the current year.

Specific Aims

Specific Aim 1: To further develop and refine our 'Dynamic Stimulus Processing' Light Model so that it can accurately predict the phase and amplitude of the human circadian system under

any lighting conditions, especially those that occur in space. This will be done using data from four completed studies of the effects on the human circadian system of: i) three-cycles of brief bright light pulses; ii) three-cycles of extended bright light pulses; iii) three-cycles of extended low- and moderate-intensity light pulses; and iv) single- and double-cycles of amplitude-suppressing critically-timed extended bright light pulses.

Specific Aim 2: To validate the Light Model refined above in Specific Aim 1 using data from four other completed studies of the effects on the human circadian system of: v) single-cycle patterns of brief bright light pulses; vi) single-cycle extended bright light pulses; vii) single-cycle extended light pulses across a wide range of intensities; and viii) sleep-wake/light-dark schedules with a wide range of periods (11-h, 20-h, 23.5-h, 24-h, 24.6-h, 28-h, 42.85-h), different light intensities during wake (1, 8, or 15 lux), and with or without a single exposure to an extended bright light stimulus (in the 11-h condition only).

Specific Aim 3: To incorporate the Light Model refined and validated above in Specific Aims 1 and 2 into our mathematical Neurobehavioral Performance Model, which will then be validated against experimental performance data collected under the wide variety of lighting conditions encompassed in the eight studies described above in Specific Aims 1 and 2.

Specific Aim 4: To develop a user-friendly Circadian Performance Simulation Software (CPSS) package that can be used to specify appropriate light schedules as a countermeasure to the poor performance and sleep quality associated with circadian misalignment in space.

v these workers, even if they work at night or on rotating schedules.

Research Progress 11/1/2003-10/31/2004:

1) We added a new component to our current circadian pacemaker model to predict the effect of non-photoc stimuli, such as a strict sleep-wake cycle, on entrainment and phase resetting of the pacemaker at low light levels. This finding suggests that the circadian pacemaker can be entrained using non-photoc stimuli and that the mathematical model can be used to simulate the effect of non-photoc stimuli on the circadian pacemaker.

2) We characterized the amplitude recovery dynamics of the endogenous pacemaker as well as identified different excitation regions of the pacemaker. The impact of this finding includes the physiological significance of the amplitude recovery dynamics of the human circadian pacemaker. The rate of recovery from amplitude reduction is slower than expected and therefore the return to equilibrium conditions make take longer. This has implications for the rate of recovery after disruption of the pacemaker (e.g., by different shift schedules or lighting conditions). Changes in amplitude of the pacemaker may affect the relative influence of the pacemaker on sleep consolidation or neurobehavioral performance during the daytime.

3) We tested our user-friendly Circadian Performance Simulation Software (CPSS) package with actual mission schedules to evaluate our predictions of performance and alertness during pre- and post- launch conditions. The impact of this is that there is now available a software package to test future predictions of the performance and alertness of crewmembers during long-duration space missions.

4) We developed preliminary algorithms for schedule assessment and countermeasure design based on the analysis of various schedules using CPSS. The impact of this finding is that the algorithm can be incorporated into our software package to assess the schedules of long duration space missions in order to design appropriate countermeasures to improve the performance and alertness of crewmembers on these missions.

5) We supported the education and work of graduate students and post-doctoral fellows.

- 6) We presented our results and software at NSBRI and NASA meetings as well as at national and international meetings.
- 7) We reported our results in abstracts and manuscripts.
- 8) All of our findings are applicable to the relevant items in the Critical Path Roadmap (6.05, 6.06, 6.21, 6.22).
- 9) We completed our specific aims 1-4. For Specific aim 4, our user friendly software – Circadian Performance Simulation Software (CPSS) - was further tested with actual mission schedules to evaluate our predictions of performance and alertness during pre and post launch conditions. In addition, we added a new component to our current version of the model to predict the response of the core pacemaker at low light levels due to nonphotic stimuli and we characterized the amplitude recovery dynamics of the circadian pacemaker.

Articles in Peer Review Journal

None

Abstracts

St. Hilaire MA, Indic P, Klerman EB, Wright KP, Kronauer RE. Addition of a Non-Photic Component to a Light-Based Mathematical Model Predicts Entrainment at Low Light Level. Association of Professional Sleep Societies, A71, 2004.

St. Hilaire MA, Kronauer RE. Determination of Endogenous Pacemaker Period may be Affected by Asymmetry in Human Photic Sensitivity. Association of Professional Sleep Societies, A71, 2004.

Premananda P Indic, Katherine Gurdziel, Richard E Kronauer, Elizabeth B Klerman. Development of Two-Dimension Manifolds for the Representation of High Dimension Mathematical Models of the Intra Cellular Mammalian Circadian Clock. Society for Research on Biological Rhythms, P222, 2004.

Dean DA, Mazza MC, Wyatt JK, Czeisler CA, Klerman EB. Circadian and Homeostatic Components of Mathematical Models of Neurobehavioral Performance for the Effects of Low Dose Caffeine During a 42-hour Forced Desynchrony Protocol. Association of Professional Sleep Societies, A77, 2004.

Presentations (Oral and Poster)

Dean D. "Circadian Amplitude and Homeostatic Components of Neurobehavioral Models for the Effects of Low Dose Caffeine during a 42-Hour Force Desynchrony Protocol", Association of Professional Sleep Societies Annual Meeting, Philadelphia, PA. 2004.

Dean D. Interactive Lecture and Computer Laboratory. Using the Circadian Performance Simulation Software to evaluate Sleep Diaries. Presented as part of the Chautauqua Short Course Program sponsored by the National Science Foundation. The lecture and lab was part of the course entitled 'Circadian Biology: From clock genes and cellular rhythms to sleep regulation'. Cambridge MA 2004.

Dean, D. Interactive Demonstration, National Space Biomedical Research Institute Workshop: Circadian and Sleep Countermeasures for Space Exploration, Society for Research on Biological Rhythms Bi-annual Meeting, (Invited), Whistler, Canada, 2004.

Dean D. "Analyzing NASA related schedules with the Circadian Performance Simulation Software", NASA-Johnson Space Center, Houston TX, 2004.

Dean D. Interactive Demonstration, National Space Biomedical Research Institute (NSBRI) PI Retreat, Demonstrated circadian related technology to NSBRI and NASA communities as a representative of the Human Performance Factors, Sleep, and Chronobiology Team, Houston TX. 2004.

Klerman EB. " Modeling circadian and sleep biology - implications for astronaut performance Invited presentation at weekly CME session for NASA Johnson Space Center physicians". Houston, TX. 2004.

St. Hilaire M. "Addition of a Non-Photic Component to a Light-Based Mathematical Model Predicts Entrainment at Low Light Level". Association of Professional Sleep Societies, Philadelphia, PA, 2004.

St. Hilaire M. "Determination of Endogenous Pacemaker Period may be Affected by Asymmetry in Human Photic Sensitivity." Association of Professional Sleep Societies, Philadelphia, PA, 2004.

Space and Earth Based Applications of Research Project

This research focuses on the further development of mathematical models and software that aid in schedule design to improve performance - and thereby public safety - for people who work at night, on rotating schedules or on non-24-hour schedules (pilots, train and truck drivers, shift workers, health care workers, etc.). This research also aids in the specification of lighting requirements aboard spacecraft and in other work conditions to insure proper entrainment and circadian phase of astronauts while in space.

Project 2:

Report for grant "Mathematical Models of Circadian/Performance Countermeasures 2004" HPF00405

Dates: Began 6/1/04

This grant was begun in the current year.

Specific Aims

Specific Aim 1: Develop and refine the current circadian, neurobehavioral performance and subjective alertness (CNPA) model with melatonin as a marker rhythm to accurately predict phase and amplitude of the circadian pacemaker. (CRL 4)

Specific Aim 2: Refine and validate the current CNPA model by using data from chronic sleep restriction protocols. (CRL 4)

Specific Aim 3: Refine the current CNPA model to incorporate wavelength of light information. (CRL 3)

Specific Aim 4: Develop Schedule Assessment and Countermeasure Design Software using the amended CNPA model from Specific Aims 1, 2, and 3 to evaluate schedules and design and test appropriate countermeasures. (CRL 5)

Progress

Specific Aim 1: We are working on a physiologically-based mathematical model of melatonin plasma concentrations. This model has physiologic outputs including time of melatonin secretion onset and offset and clearance rates. The model will then be used for simulations and for analyses of melatonin data. Results of the model will be integrated with our other circadian mathematical models, including those of performance and alertness.

Specific Aim 3: Our current light-based circadian mathematical model converts light information, measured in lux, to a drive that stimulates phase-shifting and entrainment of the circadian pacemaker. Recent studies on the effect of wavelength on melatonin suppression and phase-shifting have suggested that the peak sensitivity of circadian photoreception occurs in the short-wavelength ("blue-enriched") spectrum at ~460nm – which is considerably different from the peak sensitivity of the photopic system at 555 nm. We have developed a preliminary model structure that assumes a two-photoreceptor system with both lux and wavelength input. We modify our definition of lux in the light pre-processor of Process L by weighting the light intensity with the spectral function of the photopic system. We insert a parallel pre-processor to represent a blue-enriched photoreceptor with a peak drive at 460 nm. The individual drives produced by these two light processors are combined to obtain one drive that acts on the pacemaker. Our next step is to compare the output of this model to experimental data to obtain parameter values.

Specific Aim 4: The goal of specific aim 4 is to determine the light levels and sleep wake history that results in optimal performance of schedules involving NASA ground and flight crew. Recent results such as the design of the Mars experimental protocol and the intermittent light study have conclusively shown the efficacy of using simulations to design operational schedules and experimental protocols. However there are no systematic and operationally ready methods for using mathematical models to quickly evaluate experimental and operational schedules. We have begun work on developing a methodology for automatically generating optimal schedules. Our approach is to use evolutionary programming techniques in conjunction with the calculus of variation to determine optimal schedules. We have begun testing the optimization framework to study the effect of lighting on long haul flight and to determine the minimal lighting requirements during the proposed manned mission to Mars.

Other: At the request of the reviewers, we have also begun work on incorporating inter-individual differences into the mathematical models.

Project 7: A Model of Circadian Disruption in the Space Environment

PI: Michael Menaker, Ph.D.
University of Virginia

Research Focus: The effect of meals, melatonin, exercise, and dark pulses on circadian dysphasia

Specific aims:

1. Evaluate the effects of constant conditions and of shift work schedules on both the maintenance of circadian rhythmicity in central and peripheral structures, and on temporal synchrony among them in a transgenic rat model system;
2. Ameliorate or prevent circadian dysphasia by manipulating meal timing, melatonin administration, forced exercise and short pulses of darkness.

Menaker: A Model of Circadian Disruption in the Space Environment

A. Specific Aims

We hypothesize that the mammalian circadian system is an ensemble of independent oscillators that is coordinated by internal signals originating in the suprachiasmatic nucleus (SCN) and perhaps other brain oscillators. In order to accomplish this, these brain oscillators

must exert control over the phases of oscillators in the periphery. We assume that there is an optimal distribution of phases (a phase map) that ensures that the physiological processes regulated by the constituent oscillators will function efficiently. We further assume that when an organism is synchronized by a stable environmental cycle (e.g., light-dark cycle) phase relationships will be optimal. However, there are several environmental conditions that have been shown to disrupt normal internal synchrony. Among these, rapid shifts in the external light cycle, arrhythmic light, and irregular feeding schedules are likely to be faced by astronauts on extended space flights. The overall aim of this proposal is to identify the signals that normally maintain adaptive internal synchrony and by manipulating these signals, to restore the circadian phase map to its optimal state as rapidly as possible. We believe that the results will have direct bearing on the health of humans in space.

Specific Aim I: Using rhythms of gene expression reported by a luciferase transgene, we will identify the signals linking brain and peripheral oscillators.

We will focus on the sympathetic and parasympathetic nervous systems and on melatonin and adrenal steroids.

Specific Aim I.a.: We will lesion sympathetic neurons with 6-OHDA and surgically remove the superior cervical ganglion. We will surgically remove vagus nerve input to subdiaphragmatic structures and test the effects of these manipulations on the phase of rhythms of gene expression in the affected organs.

Specific Aim I.b.: We will study the effects of pinealectomy and adrenalectomy and of replacement with rhythmic melatonin and corticosterone on phase relationships among central and peripheral oscillators.

Specific Aim I.c.: We will validate our *in vitro* phase measurements with newly developed *in vivo* technology which enables us to measure gene expression in awake behaving animals and with *ex vivo* measurements as well. We will measure circadian rhythms of blood pressure and heart rate by telemetry to determine if rhythms in the cardiovascular system as a whole behave as do gene expression rhythms in isolated blood vessels and heart muscle.

Specific Aim II: We will evaluate potential countermeasures by exposing our experimental animals to conditions that disrupt the normal circadian phase map and then determining if the potential countermeasures are able to restore it rapidly.

AS COUNTERMEASURES WE WILL USE LIGHT CYCLES AND PULSES, RIGIDLY SCHEDULED MEALS, CYCLES OF EXOGENOUSLY APPLIED MELATONIN AND, IN PARTICULAR, DRUGS (SOME OF WHICH ARE ALREADY IN COMMON MEDICAL USE) THAT AFFECT SYMPATHETIC OR PARASYMPATHETIC FUNCTION.

Research Progress 11/1/2003-10/31/2004:

We are still analyzing and writing up the data from our extensive constant light experiments. These were collected almost exclusively during the last grant period and will be detailed in the final report for that period, and submitted for publication by the end of October. Our new grant period began on the first of July, so we have been working on it for a little over 3 months. We have been working to develop the surgical procedures that will be required to complete Specific Aim Ia (superior cervical ganglionectomy and sub-diaphragmatic vagotomy). Once we have

worked out the surgery we will assess the effects of these neural inputs on the phases of peripheral oscillators in the pineal, liver, and other visceral organs. We have pinealectomized our per-luc rats and looked for effects on the phase or robustness of rhythms in liver (Specific aim Ib), so far without positive results, but we have really just begun this work. We have also started a new series of experiments on the effects of methamphetamine on circadian rhythmicity. We have extended the well-known circadian effects of this drug to mice (most of the previous work has on rats) with the objective of using the available mouse knock-outs to clarify sites of the drug's molecular action. Initial results from these experiments appear promising.

Abstracts

Menaker M, Yoshikawa T, Davidson A (2004) The mammalian circadian axis. Society for Research on Biological Rhythms, Whistler, BC, Canada, June 24-26, 2004.

Yoshikawa T, Yamazaki S, Menaker M (2004) Per1-luc expression rhythm of central and peripheral oscillators in LL-treated arrhythmic rats. Society for Research on Biological Rhythms, Whistler, BC, Canada, June 24-26, 2004.

Presentations

Yoshikawa T, slide presentation: "Per1-luc expression rhythm of central and peripheral oscillators in LL-treated arrhythmic rats," Society for Research on Biological Rhythms, Whistler, BC, Canada, June 24, 2004.

Tataroglu O, Menaker N (2004) "Effects of chronic methamphetamine application on circadian wheel running activity in C57Bl/6J, C3H and Per1-Luc mice" Society for Research on Biological Rhythms, Whistler, BC, Canada, June 24-26, 2004.

Menaker M, invited speaker, Society for Research of Biological Rhythms symposium "Circadian System Organization: Hierarchies, Networks or Neither;" Whistler, British Columbia June 23, 2004, lecture title: "A Little of Both"

Menaker M, invited speaker, Yale University, Department of Cellular and Molecular Physiology Seminar Series, June 10, 2004; lecture title: "Circadian organization: a circus or an orchestra?"

Publications

Davidson AJ, Yamazaki S, Menaker M (2003) SCN: Ringmaster of the circadian circus or conductor of the circadian orchestra? In *Novartis Foundation Symposium 253: "Molecular*

Project 8: Circadian and Vestibular System Relationships

PI: Lawrence P. Morin, Ph.D.

State University of New York, Stony Brook

Research Focus: Three dimensional motions to stimulate the vestibular and circadian system

Specific aims:

1. Identify efferent and afferent anatomical connections between the vestibular nuclei and the intergeniculate leaflet;
2. Test the hypothesis that patterned moving light (an optokinetic stimulus) will functionally

activate the vestibular and circadian systems;

3. Test the hypothesis that a non-locomotor, non-photic three-dimensional motion stimulus will functionally activate the vestibular and circadian systems, as measured by FOS induction in the IGL and circadian phase shifts.

Research Progress 11/1/2003-10/31/2004:

Project Accomplishments

– During the period, OCTOBER 1, 2003 – SEPTEMBER 30, 2004, we published one major paper and have submitted a second. The paper is Horowitz, S.S., Blanchard, J.H. and Morin, L.P. (2004) Intergeniculate leaflet and ventral lateral geniculate nucleus afferent connections: an anatomical substrate for functional input from the vestibulo-visuomotor system. J. Comp. Neurol., 474, 227-245.

As the title indicates, this paper describes a neuroanatomical substrate that connects the vestibular system to the circadian rhythm system. It also documents the numerous brainstem nuclei containing neurons that project to the intergeniculate leaflet. The work demonstrates the existence of a route by which vestibular activating stimuli might alter circadian rhythm regulation, consistent with our original hypotheses on the matter.

While this has broad implications in and of itself, even more important has been the by-product of this research. By a curious coincidence, we were evaluating the location of cells containing the relatively novel neuromodulator, hypocretin (orexin), that project to the intergeniculate leaflet (IGL) and performing continued evaluation of both the vestibular system anatomy and the IGL. This conjunction of anatomical research has produced a second manuscript, "Medial vestibular connections with the hypocretin (orexin) system," authored by Horowitz, Blanchard and Morin. It is presently under submission.

This manuscript describes the projections of the hamster medial vestibular nucleus. While that information is not particularly novel, the fact that we were able to provide a context relative to the hypocretin system is quite novel. The hypocretin system is well known as a substrate responsible for the modulation of sleep and is essential for normal sleep behavior. Our major contribution here is the observation that the vestibular system projects to many of the same brain regions receiving hypocretin input and, further, the regions known to participate in sleep regulation receive projections from both the medial vestibular nucleus and the hypocretin neurons of the lateral hypothalamus. This strongly suggests, per earlier hypotheses, a functional relationship between the vestibular system and the regulation of sleep.

Investigation of this issue has taken an additional interesting turn with the preparation of a manuscript about to be submitted ("Descending projections of the IGL: relationship to the sleep/arousal and visuomotor systems," authored by Morin and Blanchard). This manuscript brings the evaluation of multiple systems full circle by returning to the IGL and documenting the brainstem targets of this nucleus. We have previously performed such an investigation for IGL projections to midbrain visual centers and for ascending projections to hypothalamus and basal forebrain. However, the present study re-casts those results in a new mold. Most importantly, IGL connectivity is shown to have 3 major and unusual properties: (A) Abundance - it is connected more than 100 separate brain regions; (B) Bilaterality - regions receiving projections from or which project to the IGL generally connect with the leaflet on both sides of the brain; and (C) Reciprocity - the regions receiving projections from the IGL generally send reciprocal projections back to the IGL.

These features are of probable significance with respect to a presently hypothetical function of the IGL, namely, the regulation of eye movements. In fact, we have preliminary data

from monkeys (collected in association with a colleague who studies this topic) showing that IGL stimulation modifies gaze holding in rhesus monkeys. The bilaterality characteristic, in particular, is a major clue to an IGL function requiring symmetrical neural activity. Eye movement is one of the few behaviors that fits that need.

Linked to the control of eye movements is the state of desynchronized sleep known as REM by virtue of the fact that the eyes typically move rapidly during this sleep stage. Interestingly, and obviously, convergently, the vestibular system is thought to be necessary for generation of the rapid eye movements during desynchronized sleep. The IGL, medial vestibular nucleus and hypocretin systems are convergent and all project to nuclei involved in sleep and visuomotor regulation.

The results of the two as yet unpublished studies are being presented at the Society for Neuroscience annual meeting in San Diego, October, 2004.

We previously submitted (2003) a NSBRI application to pursue the functional relationship between the vestibular system and sleep regulation. In light of our anatomical data supporting the fundamental basis for vestibular system control over sleep behavior, we have re-submitted that application with minor revisions. That research would be conducted with Dr. Fred Turek, Northwestern University, and if supported, be an active contribution to the Neurovestibular Team of NSBRI.

On the practical side, the implications of the anatomical studies for the control of sleep are significant and would be the focus of the pending NSBRI project, should it be funded. The basic premise of that application is that the vestibular input can do two things to the brain regulation of sleep: 1) sudden vestibular system activation will wake sleeping brains and 2) the appropriate gentle oscillatory vestibular stimulus will facilitate sleep in awake individuals. Envision being rudely awakened by somebody dumping you (while sleeping) out of a hammock; Or, gently rocking a fussing baby to sleep. This modal expectation of vestibular effects is the basis for our prediction that the reason for profound sleep loss in a weightless environment is the existence of erroneous signals to the brain from the vestibular gravity sensing apparatus, the otoliths. We expect that controlled manipulation of the otolith sensory function will, on the one hand, facilitate sleep, and on the other, induce wakefulness. The information acquired with respect to this topic can quickly lead to tests in the weightless environment of an orbiting spacecraft and development of countermeasures to the sleep deficit problem based on an understanding of the vestibular contribution to the sleep debt.

We have been unable to convincingly establish that there is an influence of the vestibular system on the circadian system or of the optokinetic system on the circadian system.

IV. Project Plans – This project ends October 31, 2004. We expect to continue working on the anatomy and some of the behavioral assessments not completed.

Project 9: Long-Term Exposure to Dim Light Desynchronizes the Circadian System of Rats

PI: *Gianluca Tosini, Ph.D.*
Morehouse School of Medicine

Research Focus: Use of melatonin to synchronize rhythms

Specific aims:

1. Determine the effect of long-term exposure to constant conditions on circadian rhythms.

*Research Progress 11/1/2003-10/31/2004:***I. Executive summary.**

Many biochemical, physiological and behavioral parameters exhibited by organisms show daily fluctuations and most of these daily rhythms persist in constant conditions, thus, demonstrating that they are driven by endogenous oscillators. The rhythms that persist in constant conditions with a period close to 24 hours are called circadian rhythms. One the most important aspects of space flight is the absence of geophysical 24-h cycles, which, of course, affects the overall temporal organization of the organisms. In the case of long-duration manned space flight, it is crucial to understand how the whole circadian system would react and behave in such circumstances.

We discovered that exposing rats to constant dim light for 60 days significantly affected the phase-relationship among circadian outputs in the SCN, retina and pineal, demonstrating that in these animals internal desynchronization of the circadian rhythms is occurring. We also observed that the circadian rhythm in arylalkylamine *N*-acetyltransferase (the enzyme that is responsible for the circadian rhythm in melatonin synthesis) was altered in both the retina and in the pineal gland. Our data also indicated that locomotor activity rhythm might be an unsatisfactory marker to assess the circadian status of the whole organism. Internal desynchronization has profound effects on the capability of the organisms to perform (mentally and physically) and to remain healthy. In this research proposal, we have designed a series of experiment aimed to understand the mechanisms that are responsible for the observed desynchronization. We believe that the model we have generated will be useful in to foreseeing and preventing dysfunction of the circadian system that may arise after long periods in the space environment where the normal cycle has been altered.

II. Project research objectives and activity

The primary goal of our investigation was to determine how the circadian rhythms in the SCN, retina and pineal and in other peripheral tissues (skeletal muscle, liver and heart) are affected by long-term exposure to constant dim light ($1\mu\text{W}/\text{cm}^2$).

To test our working hypothesis, we decided to investigate how circadian rhythms in the SCN (by assessing locomotor activity and *Per1* mRNA levels), in the retina and in the pineal (by measuring *Aa-nat* mRNA levels) are affected by prolonged exposure to constant dim light (CDL, $1\mu\text{W}/\text{cm}^2$). 36 rats were housed in CDL and locomotor activity was continuously monitored using a computerized system. After 0 (controls), 5, and 60 days the animals (6 individual for each time points) were sacrificed at six different circadian times (three during the light phase [CT4, CT8 and CT12] and three during the dark phase [CT 16, CT20 and 24]) and tissues or organs were collected and immediately frozen on dry ice. In order to insure that each animal was sacrificed at the exact circadian time, the following procedure was adopted. For each animal, the time of sacrifice was determined based upon each animal's locomotor activity rhythm. Circadian Time 12 (CT12) was defined as the time at which an animal began its daily bout of wheel running activity. The day before the sacrifice, running wheel records were plotted, and a line was drawn through activity onsets in order to calculate CT12 on the day of the killing. All other circadian times were calculated relative to CT12.

Running wheel activity. In all animals tested, clear circadian rhythms in locomotor activity were observed. As expected, the free-running period was longer than 24 hours (for all the animals tested the mean was 24.7 hrs, SEM = 0.07). There was no noticeable difference in the locomotor activity pattern (i.e., in the free-running period or in the duration of the activity) between the first days and the last days of recording, indicating a remarkable stability of this rhythm.

Per1 expression in the SCN. Using *in situ* hybridization we investigated the pattern of *Per1* gene expression in the SCN. *Per1* expression in the SCN showed robust rhythmicity in animals kept in LD cycles (Figure 1A, Kruskal-Wallis, $P < 0.05$), the rhythmicity persisted in animals exposed to CDL for five days (Figure 1B, Kruskal-Wallis, $P < 0.05$), and in animals that have been exposed to CDL for 60 days (Figure 1C, Kruskal-Wallis, $P < 0.05$). In all three conditions the *Per1* expression peaked during the middle of the day (subjective day for the animals held in constant conditions) around CT 4-8. The only noticeable differences between LD and after 60 days in CDL were a small reduction in the amplitude of the rhythm and an increase in the overall variability.

Aa-nat expression in the retina. In LD conditions, *Aa-nat* mRNA levels showed marked day-night variations (Fig. 2A, Kruskal-Wallis, $P < 0.05$). The retinal *Aa-nat* mRNA rhythm persisted after 5 days of CDL conditions (Fig. 2B, Kruskal-Wallis, $P < 0.05$). No significant circadian rhythmicity was observed after 60 days in CDL (Fig. 2C, Kruskal-Wallis, $P > 0.05$). The loss of circadian rhythm was due to the fact that in some animals (seven or eight) *Aa-nat* mRNA levels were found to be high during the subjective day or low during the subjective night.

Aa-nat expression in the pineal. In LD conditions, pineal *Aa-nat* mRNA levels showed a robust day-night variation (Figure 3A, Kruskal-Wallis, $P < 0.05$). *Aa-nat* mRNA levels were very low during the day and higher at night. Pineal *Aa-nat* mRNA rhythm persisted after 5 days of CDL conditions (Figure 3B, Kruskal-Wallis, $P < 0.05$); *Aa-nat* signals were observed throughout the day with highest level at CT16-20 and lowest at CT12. As observed in the retina *Aa-nat* mRNA levels were not rhythmic after 60 days in CDL (Kruskal-Wallis, $P > 0.05$). Also in this case the loss of significant difference between day and night values was due to the fact that in eight animals *Aa-nat* mRNA levels were high during the subjective day or lower during the subjective night (Figure 3C).

Our work demonstrates that long-term exposure to constant condition has profound effects on the animal's temporal organization. In particular, our data demonstrated that in some animals (20-30 %) *Aa-nat* (and thus melatonin synthesis) rhythmicity in the pineal and in the retina is significantly affected by long-term exposure to CDL, similarly *Per 1* gene expression in the skeletal muscle was found to altered by long-term exposure to CDL in some animals.

We believe that these observations are of interest for long-term space exploration, and the understanding of the mechanisms responsible for the phenomenon observed could help in preventing as well as in designing of countermeasures to avoid the occurrence of internal desynchronization.

We have participated at all the monthly teleconference meeting with the team. We participated to NASA-NSBRI retreat at in Montgomery (Texas) in January 2004. In addition, I have

organized a symposium (funded by NSBRI) entitled “*Circadian and Sleep Countermeasures for Space Exploration*” at the biannual meeting of the Society for Research on Biological Rhythms held in June 2004 (Whistler, BC, CAN).

III. Implications of project findings for future research:

The experiments that we have performed during the last year have expanded and confirmed our preliminary results demonstrating once again that internal de-synchronization may occur in some animals (20-30%) when exposed to constant dim light.

APPENDIXES

A. Project data

Figure 1. Circadian variation of *Per1* mRNA levels in SCN measured using semi-quantitative *in situ* hybridization. A) 12L:12D; B) after 5 days in CDL and C) after 60 days in CDL. The white circles represent individual values, the black circles represent the mean values ($\pm$ SEM) (N= 6 for each time point).

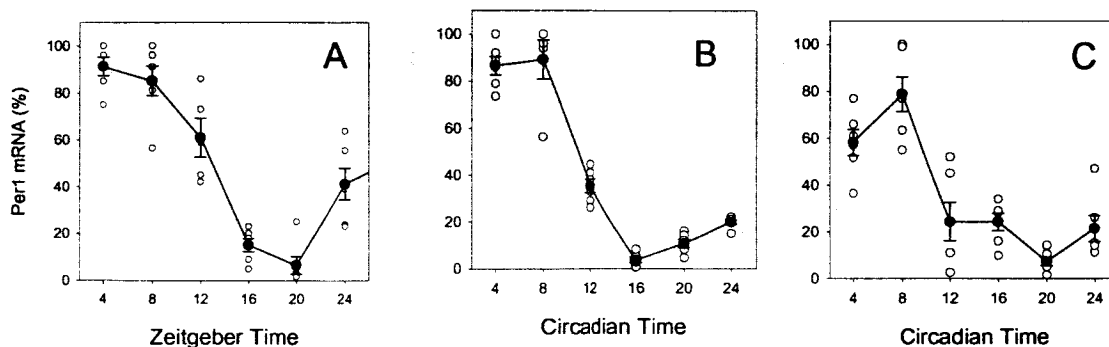


Figure 2. Circadian variation of *Aa-nat* mRNA levels in the retina. A) 12L:12D; B) after 5 days in CDL and C) after 60 day in CDL. The white circles represent individual values, while the black circles represent the mean ($\pm$ SEM) values (N= 6 for each time point). Values higher than 41 % (i.e., the maximum value recorded after 5 days in CDL during the subjective day) and lower than 60 % (i.e., the minimum value recorded after 5 days in CDL during the subjective night) were considered out of the expected range (desynchronized).

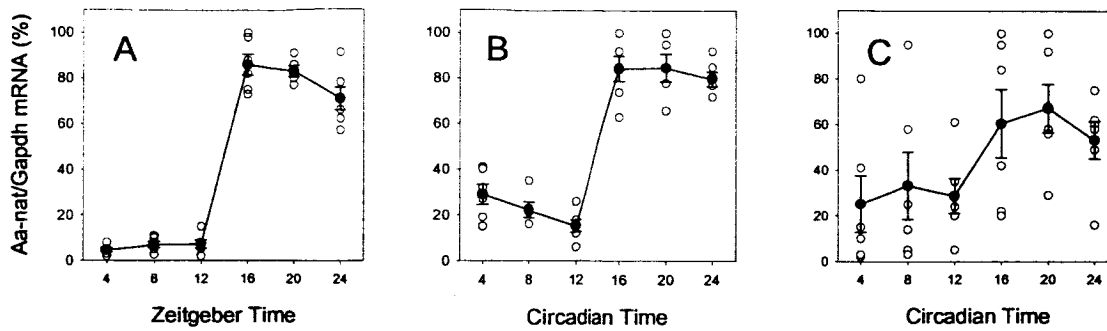
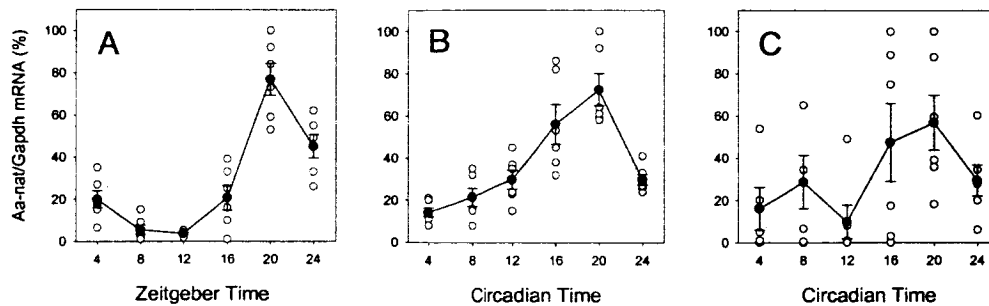


Figure 3. Circadian variation of *Aa-nat* mRNA levels in the pineal. A) 12L:12D; B) after 5 days in CDL and C) after 60 day in CDL. The white circles represent individual values, while the black circles the mean ($\pm$ SEM) values (N= 6 for each time point). Values higher than 42 % (i.e., the maximum value recorded after 5 days in CDL during the subjective day) and lower than 22 % (i.e., the minimum value recorded after 5 days in CDL during the subjective night) were considered out of the expected range (desynchronized).



B. Publication lists

Fukuhara C., Tosini G. Long-term exposure to constant dim light desynchronizes the circadian of Rats. *J. Biol. Rhythms* (Submitted, revision requested).

Tosini G., Aguzzi J. (2004). Effects of Space Flight on Circadian Rhythms. In: *Advances in Space Biology and Medicine* (Ed. G. Sonnenfeld), Elsevier (in press).

Sakamoto K., Liu, C., Tosini, G. (2004) Circadian Rhythms in the retina of rats with photoreceptor degeneration. *J. of Neurochemistry*.(in press)

Fukuhara C, Liu C., Ivanova T.N., Chan G.C-K, Storm D.R., Iuvone P.M., Tosini G. Gating of the cAMP signaling cascade and melatonin synthesis by the circadian clock in mammalian retina. *J. Neuroscience* 24:1803-1811.

Project 10: Animal Model for Sleep Loss and Circadian Disruption

PI: Fred W. Turek, Ph.D.
Northwestern University

Research Focus: The effects of chronic exposure to 12 hour periods of imposed wakefulness on sleep/wake, performance and circadian phase and the impact of countermeasures such as melatonin and exercise.

Specific Aims:

1. Determine the effects of 12 hours of imposed wakefulness during the normal active and inactive periods on circadian rhythms, the sleep-wake cycle and neurobehavioral and motor performance measures in the mouse;
2. Treatment with either a physiological or pharmacological dose of melatonin at the beginning of the imposed period of wakefulness will alter the effects of this temporal desynchrony on the circadian clock, the sleep-wake cycle and/or neurobehavioral and motor performance measures;
3. Access to a wheel (exercise) when in the home cage, will alter the effects of the imposed periods of wakefulness on the circadian clock, the sleep-wake cycle and neurobehavioral and motor performance measurements.

Research Progress 11/1/2003-10/31/2004:

The adverse effects associated with imposed disruptions of the normal circadian and sleep-wake cycles are particularly relevant to NASA personnel and their ability to carry out normal duties at a high level of efficiency. Many space travel situations demand that both ground-based and flight personnel engage in duty schedules that can lead to circadian rhythm disruption and sleep loss. The tasks that can be affected involve vigilance, operation and control of vehicles/aircraft, maintenance, preparation and operation of equipment, as well as command and control activities. Night operations are important for successful missions, and there is a clear need to find

countermeasures that can alleviate the adverse effects of these activities on human circadian rhythms and sleep as well as on neurobehavioral capabilities and on physical performance.

Despite the high prevalence of chronic partial sleep loss and circadian disruption due to shiftwork in modern society, no animal models have previously been developed to systematically examine the effects of chronic partial sleep and circadian disruption on sleep architecture and performance. The use of a new animal model, as outlined in the original proposal, will lead to new insights into how the circadian and sleep systems are affected by the disruption of their normal phase relationship to one another, and how this temporal disorganization influences neurobehavioral capabilities and motor performance. Information gained using this novel animal model will also be important in the development of effective countermeasures to the adverse effects associated with circadian disruption and sleep loss. These countermeasures could be useful in a number of situations involving NASA personnel, particularly in extended duration space flight missions that will result in challenges to the sleep and circadian system of the flight crew and support teams. This project will also provide important insights in to the interactions between the circadian and sleep/wake systems.

There are three specific aims of the project 1) to determine the effects of 12 hours of imposed wakefulness during both normal active and inactive periods on circadian rhythms, the sleep-wake cycle and neurobehavioral and motor performance measurements 2) to test the hypothesis that treatment with either a physiological or pharmacological dose of melatonin at the beginning of the imposed period of wakefulness will alter the effects of this temporal desynchrony on the circadian clock, the sleep-wake cycle, and/or on neurobehavioral and motor performance measurements, 3) to test the hypothesis that access to a wheel (exercise) when in the home cage, will alter the effects of the imposed periods of wakefulness on the circadian clock, the sleep-wake cycle, and/or neurobehavioral and motor performance measurements.

During the award period we have examined the impact of chronic partial sleep loss and circadian disruption on sleep, circadian rhythms and neurobehavioral and motor performance. With the development of an animal model of sleep loss and circadian disruption we have determined that mice respond in a similar way to chronic partial sleep loss and circadian disruption as humans. Sleep is altered depending on the strain and the time-of-day of sleep restriction. During our forced wakefulness procedure animals are not able to get any REM sleep but can get anywhere between 5 to 40 % NREM sleep. Over the 10 day period of partial sleep restriction animals are sleep deprived of between 26 to 41 hours of sleep, depending on the strain and time of sleep restriction (i.e. light or dark period). The degree of sleep loss seen in these studies is equivalent to a human obtaining approximately 5-6 hours of sleep per night, which is commonly seen on shuttle missions. We have also determined that this moderate degree of sleep deprivation does not significantly impair performance on a task of neurobehavioral and motor performance. The data collected during this unique study appears to be similar to data recently published on human subjects exposed to chronic partial sleep loss.

Synergism between team projects or with other teams.

There is considerable synergism between studies in the Human Performance Factors, Sleep and Chronobiology Team. As a team we are able to use information that is gained from one project to improve others within the team. The exchange of knowledge is augmented by participation in monthly teleconferences by all principles investigators in the team. These teleconferences serve to keep all team members apprised of new and exciting findings by their fellow team members. It also serves as an opportunity to discuss these findings and the conclusions that can be drawn from them. In addition, members of the team have opportunity to meet at events like the annual Association of Professional Sleep (APSS) Society meeting and upcoming Society for Research on Biological Rhythms meeting were a workshop on NASA and NSBRI is being held.

This project has considerable synergism with other projects and members of this team. For example work being conducted by Dr Dinges in human subjects examining the impact of chronic partial sleep loss on sleep, rhythms and performance is showing similar results to those observed in our animal model of sleep loss. Furthermore the data from the experiments described above with provide useful information for the refining mathematical models of sleep-wake.

Publications

1. Woods B. C., Reid K. J., Losee M. W., Laposky A. D., Turek F. W. (2004) Animal Model for Shiftwork Resulting in Chronic Partial Sleep Loss. Sleep. 27 (Abstract Suppl.): A164.
2. Losee M. W., Woods B. C., Reid K. J., Turek F. W. (2004) Performance on an Active Avoidance Task during Sleep Loss and Circadian Disruption. Sleep. 27 (Abstract Suppl.): A165.
3. Reid K. J., Zee P.C. (2004) Circadian Rhythm Disorders. Seminars in Neurology. 24(3): 315-325.

Immunology Infection and Hematology-Annual Team Report

Team Leaders:

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List of Team Project Titles and Principal Investigators

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Effect of Deep Space Radiation on Human Hematopoietic Stem and Progenitor Cell Function

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Apoptosis and Immune Homeostasis During Hindlimb Unloading

P.I. – Yufang Shi, Ph.D., Department of Molecular Genetics, Microbiology and Immunology, Robert Wood Johnson Medicine School, 675 Hoes Lane, Piscataway, New Jersey 08854, Telephone: 732-235-4501, Fax: 732-235-4505; Email: shiyu@umdnj.edu

Biology of Virus Infections: Radiation and Immunity

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Bed Rest and Immunity (NASA Bed Rest Study Campaign 1).

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Space Flight Immunodeficiency

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Suspension, the HPA Axis and Resistance to Infection

P.I.-Gerald Sonnenfeld, Ph.D., MSM., Vice President for Research, Binghamton University, State University of New York, Vestal Parkway (Courier address), P.O. Box 6000 (Mail address), Binghamton, New York 13902-6000, Telephone: 607-777-4818, Fax: 607-777-2501, Email: sonneng@binghamton.edu

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Abstract

During the period from October 1, 2003-September 30, 2004, there were major changes in the Immunology, Infection and Hematology Team. Dr. Ann R. Kennedy became the new Team Leader, replacing Dr. William Shearer, and Dr. Alan Gewirtz became the new Associate Team Leader, replacing Drs. Sonnenfeld and Butel as the previous Associate Team Leaders. Two projects ended during this period; the Principal Investigators (PIs) for these projects were Dr. William Shearer and Dr. Gerald Sonnenfeld. Three projects were competitively reviewed and renewed such that the work of these projects is continuing into the next year; the PIs for these projects are Dr. Janet Butel, Dr. Alan Gewirtz and Dr. Yufang Shi. Two new projects in the IIH Team have begun; the PIs for these projects are Dr. Ann Kennedy and Dr. Gerald Sonnenfeld. The new project for Dr. Gerald Sonnenfeld is a human bed rest study in which Dr. Butel and Dr. Shearer are participating as Co-Investigators.

The aims of the continuing Butel grant, are: (1) To determine the effects of space radiation and hind limb unloading on host control of virus infections and virus-induced cancers, and to develop countermeasures to minimize adverse effects of virus infections; and (2) To characterize direct effects of radiation on viruses and virus-infected cells. These approaches address the hypothesis that conditions of space flight, including solar radiation, will damage the human immune system, leading to reactivation of latent viruses, increased viral infections and disease, and development of cancer. The aims of the current Gewirtz grant are as follows: 1) Determine the cellular and molecular consequences of exposing human hematopoietic stem cells to an environment that simulates the radiation environment of deep space, and 2) Design potential countermeasures to obviate or negate cellular and molecular damage identified during the course of carrying out the above aim. Dr. Shi's project is to elucidate the effect of hindlimb unloading, a murine model simulating the effect of spaceflight, on the immune system. The specific focus of this project is to examine lymphocyte apoptosis and alterations in immune response. The primary aim of Dr. Kennedy's project is to determine whether a dietary supplement containing L-selenomethionine, vitamin E succinate, N-acetyl cysteine, vitamin C, Co-enzyme Q10 and alpha-lipoic acid (reduced form) can prevent space radiation induced myeloid leukemia. As part of this project, the role of the oxidation resistance gene (OXRI) in protection against radiation induced oxidative stress will be determined in selected populations of white blood cells. Dr. Sonnenfeld's new grant within the IIH team involves studies on the effects of bed rest on the immune system; the work of this grant will be carried out at the MEDES facility in Toulouse, France and the University of Texas.

Two grants have ended this past year. The goals of Dr. Shearer's grant which ended this past year were as follows: Use the mouse polyomavirus space radiation model to determine if those immune mechanisms that become compromised permit reactivation of latent virus infection and development of malignancy and initiate cytokine pretreatments to mitigate radiation damage and its effects. The hypothesis for Dr. Sonnenfeld's project which ended this past year was that antiorthostatic (AOH or hindlimb) suspension of mice, a model for some of the effects of space flight on the immune system, results in altered resistance to infection with pathogens. Testing of this hypothesis provided data to allow development of future studies to determine whether space flight affects resistance to infection and if countermeasures can be developed to prevent any detrimental effects.

II. INTRODUCTION

A) The Team Activities and Interactions between Team Members

The IIH Team has been a very interactive team, with numerous projects involving Drs. Shearer, Butel and Sonnenfeld, as reflected in the past year's publications. This team of investigators is involved in a new grant involving a human bed rest study; the P.I. of this new grant is Dr. Sonnenfeld. This is a grant to support studies on the effects of bed rest on the immune system carried out at the MEDES facility in Toulouse, France and the University of Texas. These are long-term studies to determine the effects of bed rest on leukocyte blastogenesis, cytokine production, chronic virus shedding, and immunization to an innocuous antigen. The human trial in France will begin in February of 2005. The arrangements to do this study included obtaining human studies approval in France, arranging for the use of laboratory resources in France and also negotiating with other investigators on the project and the project team to assure that sufficient blood, urine and saliva samples could be obtained to allow all assays to be performed. For the Texas bed rest study, the initial experiments have begun. Three subjects have been placed in the study, and blood, urine and saliva samples have been obtained from them (2 pre-bed rest and 1 during bed rest to date). It has been shown that the assays to be used in the French bed-rest study are functional and that there will be analyzable data when the current bed rest campaign is completed after 60 days of bed rest. The purpose of this project is to conduct studies, under strictly standardized conditions, to lead to the development of countermeasures to the adverse physiologic effects of space flight for humans. The purpose of the Sonnenfeld component of the study is to examine the effects of space flight conditions using the bed rest model on immunity and infections. The aims of the Butel part of the study are to detect and quantitate reactivation and shedding of herpesviruses and polyomaviruses in the study volunteers. This human bed rest study was conceived on the basis of data obtained by IIH Team members from their previous laboratory investigations. Studies such as these will help in the development of countermeasures for space flight induced changes in the immune system.

Dr. Butel also has a current basic science grant with focus on human herpesviruses and polyomaviruses. These viruses, known to cause human disease, are reactivated and undergo increased replication in humans under space flight conditions. Dr. Butel has recently observed that radiation can also cause reactivation of latent viruses and the extent to which radiation can interact with other space flight conditions will be the subject of her future work, along with the development of countermeasures to prevent the reactivation of latent viruses during space travel. The specific aims of the project are: (1) To determine the effects of space radiation and hind limb unloading on host control of virus infections and virus-induced cancers, and to develop countermeasures to minimize adverse effects of virus infections; and (2) To characterize direct effects of radiation on viruses and virus-infected cells.

Other current team members have research grants focused on basic laboratory research. The work of Dr. Alan Gewirtz has shown that human hematopoietic stem cells are extremely sensitive to the cytotoxic effects of HZE particles, which represent the major type of radiation of concern for the health of astronauts during space travel, as well as other types of ionizing radiation, with doses of radiation comparable to those received by astronauts now on the International Space Station having significant cell killing capabilities. These stem cells are the ultimate source of both the blood and immune system and damage to these cells can have grave immediate as well as long term consequences. Dr. Gewirtz is concerned that there could be highly significant deleterious effects on the ability of the hematopoietic stem

cell pool to survive and proliferate following exposure to the radiation doses expected during a Mars voyage, and that a complete failure of hematopoiesis (aplastic anemia) may occur in the astronauts. The continued work of Dr. Gewirtz is focused on determining the conditions under which aplastic anemia can occur during extended space travel and the development of countermeasures to prevent its occurrence. As part of the NSBRI work of Dr. Gewirtz, Dr. Betsy Sutherland from the Brookhaven National Laboratory is evaluating the types of DNA damage induced by space radiation in stem cells. The longer-term consequence of damage to the stem cells is the induction of malignancies of hematopoietic origin, which is another area of concern to the IIH team.

The work of Dr. Ann Kennedy is focused on countermeasures for space radiation induced myeloid leukemia. It is now well established that ionizing radiation can induce malignancies in hematopoietic cells at the radiation doses that astronauts traveling to Mars are expected to receive. In fact, even the doses received by some astronauts in previous missions, including current trips to the International Space Station, are within the range of doses that have been shown to result in statistically significant increases in the leukemia incidence rates in certain human populations. Radiation induced leukemia can occur within a few years of radiation exposure, thus, leukemia could occur during the time of a mission to Mars. It is expected that an increase in the leukemia incidence rate will be observed at considerably lower radiation doses than those already associated with increased leukemia incidence rates, as there will be exposure to radiations with increased relative biological effectiveness (RBE) values during space travel and astronauts will receive continuous space radiation exposures which can act as a promoting agent for the development of malignancies. Potential countermeasures for the biological effects of space radiation have been identified by the Kennedy laboratory in a project previously funded by the NSBRI; these countermeasures include non-toxic dietary concentrations of the following agents: L-selenomethionine, vitamin E succinate, N-acetyl cysteine, vitamin C, Co-enzyme Q10 and alpha-lipoic acid (reduced form). The work of the Kennedy laboratory is focused on the use of these agents to inhibit radiation induced myeloid leukemia and affect surrogate endpoint biomarkers of leukemia development. Dr. Kennedy has collaborative projects with Drs Gewirtz, Sutherland, Shi and Sonnenfeld.

By employing hindlimb suspension in animal studies, the laboratory of Dr. Yufang Shi has observed that lymphocyte numbers are dramatically reduced and the immune response significantly altered under these conditions. Dr. Shi has also observed that hindlimb unloading and radiation synergistically increase cell killing (by apoptosis) in lymphocytes and it is expected that lymphocyte apoptosis mediates space flight-associated immune modulation. Countermeasures for these significant effects will continue to be a focus in this laboratory. It has already been observed that the immunoresponsiveness of T lymphocytes and the absolute lymphocyte numbers, lymphocyte blastogenic capability and eosinophil percent in the peripheral blood of astronauts are depressed postflight. Similar changes have been observed in animals postflight and it has been assumed that hypoplasia of the lymphoid organs in animals postflight reflects the drop in lymphocyte numbers during space travel. Dr. Shi has collaborative projects with Dr. Gerald Sonnenfeld and with Dr. Ann Kennedy.

In addition to a reduction in lymphocyte numbers, numerous other changes have been observed post-flight in astronauts which suggest that infectious diseases are likely to be a

problem for astronauts. As part of the Sonnenfeld grant work completed this past year, it was shown that that conditions simulating space flight (utilizing the animal hind-limb suspension model system) decreased the resistance of animals to infection with *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and EMC virus.

The hypothesis being addressed in the work of Dr. Shearer's laboratory was that space flight radiation will suppress the human immune system leading to reactivation of latent viruses, increased viral infections and disease, and the development of cancers. Since this hypothesis could not be tested in humans, a murine animal model of radiation and latent polyomavirus (PyV) infection was used to determine the harmful effects upon the immune system with reactivation of latent virus infection.

B) Team Risk Areas and Enabling Questions

1) Dr. Janet Butel's grant is focused on the following Critical Path Roadmap (CPR) Risks: Risk #8 (Immunodeficiency/Infection); Risk #9 (Virus-Induced Lymphomas and Leukemias, Risk #11 (Altered Host-Microbial Interactions), Risk #31 (Carcinogenesis). The specific Critical Questions addressed are: 8a, 8b, 8c, 8d, 8e, 8f, 9a, 9b, 9c, 9d, 9e, 9f, 9g, 11c, 11e, 31a, 31b, 31e, 31f, 31g, 31h.

2) Dr. Shi's project addresses CPR Risk 22: Immunodeficiency/Infections. Critical Questions Addressed: 7.03, 7.10

3) Dr. Gewirtz's project addresses CPR area 10 – Anemia, blood replacement and marrow failure.

4) Dr. Kennedy's project addresses the following CPR Risks: Risk 23 (Carcinogenesis Caused by Immune System Changes), Risk #22 (Immunodeficiency/Infections); Risk #7 (Inadequate Nutrition [Malnutrition]), and Risk#38 (Carcinogenesis Caused by Radiation). CPR questions addressed: 5.03, 7.15 7.24, 7.26, 10.03.

5) Dr. Sonnenfeld's project which ended this past year focused on the following CPR Risk(s): 22, Immunodeficiency; 26, Altered Host-Microbial Interactions. Critical Questions Addressed: 7.01, 7.03, 7.10, 7.11, 7.19, 7.22, 7.23, 7.26, 7.29.,

6) Dr. Shearer's project which ended this past year addressed the following CPR Risks Addressed: 8 Immunodeficiency/Infection; 9 Virus-Induced Lymphomas and Leukemias; 31 Carcinogenesis. Enabling Questions Addressed: 8a, 8b, 8c, 8d, 8e, 9a, 9b, 9c, 9d, 9e, 9f, 31a, 31b, 31f, 31g, 31h.

C) Ways in which the Team Supports the Vision for Space Exploration

The Vision for Space Exploration aims to conduct research "to ensure the health and safety of astronauts during long-duration space exploration far from Earth. We are actively engaged in promoting new approaches that will substantially involve industry and universities in these efforts. . . . focused on crew health and life-support systems, countermeasures and radiation protection." The IIH team research is consistent with several of these goals. All of the projects involve countermeasure development for IIH health problems identified during space travel, and they all involve university faculty members highly skilled in their respective areas of expertise. The research performed as part of Dr. Butel's and Dr. Shearer's grants is aimed at mitigating the immunosuppressive effects of space radiation and latent virus reactivation, which can lead to chronic viral infection and cancer of the immune system. The current Shi, Butel, Gewirtz and Kennedy projects can be thought of as involving experiments in radiation protection, and the Sonnenfeld human bed-rest studies will be directly applicable to concerns about crew health in during space travel.

III. TEAM ACCOMPLISHMENTS

A. Key Findings

As the new grants for **Drs. Sonnenfeld and Kennedy** have only been funded for a few months (these grants began on July 1, 2004, and this report is being written on October 20, 2004), there are no key findings at this point for either of these new projects. For both projects, work has begun to address the research areas described in the grants. The projects that have been funded for a longer period of time are summarized below.

1) Dr. Butel. The main findings of the work by Dr. Butel include the development of a mouse polyoma virus space radiation model, the development of a sensitive and specific real-time quantitative polymerase chain reaction assay for the detection of mouse polyoma virus DNA sequences, the demonstration that the mouse anti-orthostatic hind limb unloading model did not affect immunity to rotavirus, a gastroenteritis virus, and the identification of a herpesvirus EBV latent protein that might modulate the development of EBV-associated disease. Specific details follow: 1) a mouse polyoma virus space radiation model was developed. Optimal doses of viral inocula, mouse target organs to analyze, optimal times of harvest of specimens, kinetics of viral clearance/entry into latency, and tissue processing methodology were all determined; a conventional PCR assay for the virus was developed. It was observed that whole-body gamma irradiation delayed clearance of primary virus infection and reactivated latent viral infections. Radiation reduced spleen cell counts and lymphocyte proliferation potential and blunted gamma interferon production (a cytokine important in controlling viral infections and cancer). These findings suggest that combined effects of radiation and virus infection on the immune system lead to immunosuppression and latent virus reactivations. 2) a sensitive and specific real time quantitative polymerase chain reaction (RQ-PCR) assay was developed for the detection of DNA sequences of mouse polyoma virus (PyV) using the ABI Prism 7000 Sequence Detection System. A quantitative assay to measure the single copy mouse p53 gene was also developed in order to normalize viral gene copy numbers to cell numbers. This RQ-PCR assay reproducibly detects 10 viral genome copies per reaction and is about 1000-fold more sensitive than the conventional PCR protocol for detection of viral sequences. A manuscript is in preparation describing this new assay. 3) The mouse anti-orthostatic hind limb unloading (HLU) model mimics some changes observed in space flight conditions. The effect of space flight conditions on mucosal immune responses using this model were determined. HLU of mice did not alter clearance of a primary rotavirus infection compared with controls or protection from rotavirus challenge 42 days later. Generation of virus-specific fecal antibodies was not significantly different between any of the groups. These data indicate that rotavirus immunity was not impacted in this model. However, gravity is not the only factor in space travel that can affect immune responses; further animal studies are needed to model the effects of multiple factors, including radiation, on immune responses. 4) data analysis and publication of studies from the previous NSBRI funding period that focused on ground-based models for immune status changes and latent virus reactivations were continued. In a study involving 70 HIV-infected individuals herpesvirus EBV viral loads in the blood and saliva as well as CD4 counts were determined. Viral loads were higher in HIV-infected patients than in uninfected volunteers and the frequency of EBV detection in blood was more frequent in patients with lower CD4 counts among HIV-infected individuals. These findings were similar to previously published observations of increased urinary excretion of polyomavirus JCV by HIV-infected patients. This study showed the crucial role that host immune function plays in regulation of latent and persistent viral infections. As even modest depressions in immune function correlated with virus reactivation and shedding, this emphasizes the importance of understanding the effect of long-duration space flight on the host immune system. 5) Collaborations continued with

Dr. D. Walling on herpesvirus EBV genotypes and function. The expression of an EBV latency-associated gene (EBNA-2) was identified as an important cofactor associated with the pathogenesis of oral hairy leukoplakia (HLP), an EBV-associated disease commonly found in immune-compromised (HIV-infected) individuals. A recombinant variant of EBNA-2, consisting of only the amino-terminal 58 amino acid residues, might modulate the pathogenesis of HLP, through modification of normal EBNA-2 protein function. Inhibiting EBNA-2 function through this protein domain might be the basis for novel pharmaceuticals against EBV-associated diseases that arise against an immunosuppressed host background.

2) Dr. Gewirtz In initial studies of the Gewirtz grant, carried out in collaboration with Betsy Sutherland's group at BNL, it was determined that human CD34⁺ bone marrow cells are very sensitive to even low doses of high-LET radiation (as low as 15-30 cGy), including Fe, Ti, Si, and protons. Because the main causes of complex damage to DNA from such high energy particles are either direct ionization of DNA or the generation of free radicals from the ionization of water, it was hypothesized that treating CD34⁺ cells with compounds that have the capacity to either alter the availability of DNA to these damages, or to scavenge the free radicals generated would be effective in reducing the overall lethality of high-LET radiation. Based on the expected damage types, two types of radioprotective agents were selected: one with potential electron-donating ligands that have been shown to localize to the necessary cellular microenvironment, and one without the ability to specifically localize. These are, respectively, Pro-methyl Hoechst and EUK-134 (Manganese derived derivative of salen-ring complexes). Both have known electron donation (catalytic scavenging) ability which should, in theory, decrease the amount and complexity of DNA damage induced by radiation exposure. Before undertaking these studies, we first carried out toxicity studies with normal CD34⁺ cells to identify maximally tolerated doses of each of these agents. We observed <19% loss in cell viability when cells were exposed to < 2 μ M PMH for 1 hr. Doses above this concentration, or exposure for greater periods of time, resulted in unacceptable toxicity. EUK-134 in the dose range tested (i.e. 5-100 μ M) was relatively non-toxic with loss of only 10-20% viability. With the establishment of acceptable treatment doses, we exposed primary CD34⁺ cells to both agents prior to irradiation with either high linear energy transfer (LET) radiation (i.e., HZE particles), or low LET ionizing radiation. Control cells were irradiated in the absence of the putative protective agents. The colony-forming abilities of control and "protected" irradiated cells were measured, as well as the presence and number of double strand breaks (DSB). DSB were detected by measurement of strand-break activated, phosphorylated histone (H2A.X) using fluorescence activated cell sorting with an FITC anti-H2A.X antibody. Levels of H2A.X were measured at 2-hr post irradiation, as its levels were reported to occur maximally during this period through DSB dependent DNA-PKcs activation. We also attempted to document the existence of repair proteins unique for repairing lesions of this nature in CD34⁺ cells, such as Ku70/80, MRE11, and Rad50 in order to assess their relevance to countermeasure development. The radioprotective effects of CD34⁺ cells pretreated with PMH or EUK-134 prior to exposure to a low-LET (¹³⁷Cs) radiation source were determined first. In cells irradiated without radioprotectant pretreatment (50-70 cGy), erythroid CFU and CFU-GEMM were reduced ~60% compared to unirradiated control cells. CFU-GM were decreased ~23-40%. Exposure to 30 cGy decreased the colonies formed in all CFU assays tested ~20-38%, except for CFU-GEMM colonies, suggesting that CD34⁺ cells exhibit a dose-dependent sensitivity to low-LET radiation. Exposure to 15 cGy reduced BFU-E colony formation by 44%, but had little

apparent toxicity to other CFU. In contrast, when cells were pre-treated with 2 μ M PMH for 1 hr, CFU survival was dramatically improved, with a loss of only 0-10% from exposures of 15-50 cGy. The decrease in CFU-GM was reduced to 30%, CFU-E to <10% and CFU-GEMM virtually to zero. This increase in cell survival was highly significant when compared to reductions up to 60% in the case of untreated cells. The PMH was unable to increase the survival of CD34⁺ CFU-E and CFU-GM colony formation when irradiated with 70 cGy. That all CFU assays tested were not equivalently sensitive to low-LET radiation, suggests that some lineage specific progenitors are more resistant to damage than others. The radio-protective effects of EUK-134 were then evaluated alone, or in combination with PMH when cells were exposed to low-LET radiation from ¹³⁷Cs. Treatment with EUK-134 alone resulted in pronounced radioprotection at doses as low as 15 to 30 cGy, leading to the recovery of colony formation in CFU-E, CFU-GM, and CFU-GEMM assays, whereas untreated cells displayed a 50-60% decrease in colonies formed when compared to unirradiated control cells. When doses were increased to 50 cGy, the protective abilities were retained in all CFU assays, with the exception of BFU-E. Above 70cGy, protection was lost in all lineages. The combination of PMH and EUK-134 restored colony formation in cells exposed to 15 cGy to that observed in unirradiated control BFU-E and CFU-E, and to 94% of unirradiated controls in CFU-GEMM. These effects were additive when compared to the protective effects of PMH alone over the range of 15 –50 cGy. Above this dose, protection was gradually lost. The ability of PMH to protect CFU in CD34⁺ cells exposed to high-LET particles was also studied. Both Fe and Ti particles displayed similar inhibition of colony formation at 50 cGy. The sensitization by protons was almost negligible in the case of CFU-E & GM at 15 cGy. Comparatively, Ti particles more effectively sensitized the CFU-E & CFU-GM, showing a 70% decrease at this dosage. BFU-E were unaffected. The decrease in colony forming abilities however was reasonably similar from 30 to 70 cGy (45-90%) between ⁵⁶Fe & Ti. BFU-E colonies were significantly decreased in ⁵⁶Fe exposed cells, as compared to Ti and Protons. All particle types, however, have similar effects in decreasing the colony formation abilities above the dosage of 50 cGy, except in case of CFU-GM by ¹³⁷Cs. These findings suggest the ⁵⁶Fe is more sensitizing than Ti and protons at low doses and also, relative biological effects (RBE) are similar irrespective of radiation source (i.e. low-LET or high-LET) above 50 cGy. PMH pretreatment protected CD34⁺ cells from damage by high-LET Fe particles up to 30 cGy. Above 70 cGy, colony formation was impaired to levels of cells un-treated with PMH except CFU-GEMM. At the dosages of Fe particles where PMH has a pronounced protective effect (<30 cGy), phosphorylated H2A.X levels were decreased, suggesting that one mechanism of protection may be through the minimization or prevention of double-stranded DNA breaks. The DSB created by high-LET particles are considered deleterious because they generate DNA ends (5'OH and 3'PO₄) that are not suitable substrates for DNA repair enzymes. In order to assess the role of PMH and EUK-134 in simplifying these DSB by facilitating their reversal to 5'-PO₄ and 3'PO₄, the dUTP (FITC) terminal transferase assay which detects 3'OH was carried out. In this way, changes in the nature of the strand break ends generated by high-LET particles were indirectly measured. If indeed PMH can function by reducing complex, unrepairable DNA strand breaks, an increase in terminal transferase positive ends should be detected by antibody staining. The PMH treated cells displayed more intense dUTP-transferase fluorescence than untreated irradiated cells, when irradiated at doses of 50 and 70 cGy. Cells pretreated with PMH and EUK-134 displayed an even more enhanced staining than PMH

alone. This suggests these agents may be playing a role in the simplification of DNA damage, which leads to the recovery of colony formation in the biological assays used for these studies. Finally, the protective effect of PMH and EUK-134 on the expression of proteins unique for DSB repair (also known as non-homologous end-rejoining) was examined. These were examined by immunostaining of fixed cells, after various pre-treatment times (without irradiation). The end processing nuclease MRE11, helicase Ku70/80, and Rad50 were localized in intensely staining nuclear foci. It has been observed qualitatively that prolonged incubation of these agents for > 20 to 60 min significantly reduces the basal nuclear levels of MRE11 and Ku70/80. However, changes in the levels of Rad 50 were uncertain. This suggests that the agents that were utilized for radio-protection may reduce the recruitment or enhance their translocation from nucleus to cytoplasm, making them unavailable in optimum concentration for repair. This finding has been strongly correlated with fact that the PMH sensitizes cells to radiation at higher concentrations or when its treatment was prolonged in other cell models. It is hypothesized that the 60 min pre-incubation times of PMH may be responsible for relative loss of radio-protection at dosages of 50-70 cGy. We conclude that PMH and EUK-134 is effective against both low and high-LET, and that similar radioprotective mechanisms may be triggered in cells by each. Experiments to address the precise mechanisms of radioprotection by these compounds are ongoing in our labs, and will hopefully assist in the development of even more efficient radioprotective agents.

3) Dr. Sonnenfeld (the grant which ended during the past year): 1) In the past year, research has concentrated on the development of an Active Hexose Correlated Compound (AHCC) as a potential countermeasure for space flight-induced immune dysfunction. AHCC is a nutritional supplement that is extracted from basidiomycete mushrooms that have been shown to enhance immune responses. It is available "over-the-counter" and has been shown to be innocuous. The studies in the past year have concentrated on the mechanism of the effects of AHCC on the immune system. 2) It was observed that treatment with AHCC restored resistance to infection in mice maintained under hindlimb-unloading conditions. The present study was designed to clarify the mechanisms by which AHCC enhances resistance to infection in this model. It was hypothesized that oral administration of AHCC would enhance the function of the immune system, which could lead to the increased resistance to infection observed in this model. AHCC or the excipient (phosphate buffered saline) was orally administered to mice and the function of the immune system was assessed in spleen and peritoneal cells isolated from those groups. The results of the current study showed that, administration of AHCC for one week prior to and throughout the 2nd day of hindlimb-unloading period enhanced the function of the immune system assessed by spleen cell proliferation and cytokine production in spleens and nitric oxide and cytokine production in peritoneal cells. In fact, AHCC restored the function of the immune system, which was severely suppressed by hindlimb-unloading conditions, to values very close to values obtained from mice under normal control conditions. Therefore, while enhancement of the immune response in normal mice may have minimal beneficial effects on resistance to infection, restoration of the immune response to normal levels in immunosuppressed hindlimb-unloaded mice appears to be crucial for their survival. In summary, the Sonnenfeld grand has shown that space flight conditions, including fluid redistribution, affect the immune system and resistance to infection and that administration of a nutritional supplement (AHCC) can prevent the development of immune dysfunction and protect against development of infection. The above results suggest that AHCC may be effective as a

countermeasure to prevent the development of immune dysfunction in astronauts and cosmonauts exposed to space flight conditions.

4) Dr. Shi – The main research findings are as follows: 1) $CD4^+CD25^+$ Regulatory T Cells are Resistant to Hindlimb Unloading. In the last reporting year, it was observed that subjecting mice to hindlimb unloading (HU) induced significant reductions in both thymocyte and splenocyte numbers. Administration of opioid antagonists or interference with the Fas-FasL interaction prevented HU-induced reductions of splenocytes, but not thymocytes. On the other hand, steroid receptor antagonists blocked lymphocyte reductions in both spleen and thymus. A detailed analysis of splenocyte populations in mice subjected to hindlimb unloading was performed. Balb/c mice were suspended for 2 days and changes in various lymphocyte populations were analyzed by staining with antibodies against specific lymphocyte surface markers: CD19, CD3 and CD56 to identify B cells, T cells and NK cells respectively, and CD4, CD8 and CD25 to distinguish T cell subpopulations. As previously observed, B cells are most significantly affected, although all major populations are reduced. It was observed that the percentage of $CD4^+CD25^+$ regulatory T cells in HU-treated mice significantly increased, while the absolute numbers were actually unchanged. Thus, it was concluded that $CD4^+CD25^+$ regulatory T cells are resistant to reduction in numbers by the stress of hindlimb unloading. 2) Role of $CD4^+CD25^+$ Regulatory T Cells in Regulation of the Proliferation of T Cells from Hindlimb Suspended Mice. It is well-known that stress can significantly influence immune reactions. The effect of hindlimb suspension on anti-CD3-induced T cell proliferation *in vitro* was examined. Equal numbers of viable splenocytes isolated from control and stressed mice were activated in 96-well plates and their proliferation was assessed by tritiated thymidine incorporation. A significant reduction in the proliferation of T cells from mice subjected to 2-days of hindlimb unloading was observed. When $CD4^+CD25^+$ regulatory T cells were selectively eliminated *in vitro* using anti-CD25 antibody, the proliferation of T cells from stressed mice recovered almost to the same level as controls. Thus $CD4^+CD25^+$ regulatory T cells are critical in down regulation of the immune response after hindlimb unloading. 3) *In vivo* Depletion of $CD4^+CD25^+$ Cells Diminishes Stress-Induced Lymphocyte Reduction. $CD4^+CD25^+$ T cells have been shown to play a central role in regulating immune responses and thus are termed regulatory T cells. Although much is known about the role of these cells in the suppressing immune responses, little is understood about the function of $CD4^+CD25^+$ (Treg) cells in the maintenance of homeostasis in lymphocytes. It was observed that *in vivo* depletion of $CD4^+CD25^+$ Treg cells by administration of anti-CD25, dramatically reduced HU-induced lymphocyte reduction. This novel finding leads to the conclusion that $CD4^+CD25^+$ Treg cells play a critical role in lymphocyte homeostasis under situations of stress. 4) HU Significantly Increases Specific Serum Proteins. It has always been difficult to quantify the degree of stress experienced by an individual since there is no specific stress marker. Proteomics technology was employed and the serum protein composition of mice before and after HU was compared. It was observed that HU consistently increased the serum level of transferrin many fold. A few other proteins that are increased by HU are currently being characterized. It is believed that further studies on these proteins may provide a marker for the measurement of stress. 5) Radiation Synergizes with HU in Depleting Lymphocytes and Reducing the Colony Formation Capacity of Bone Marrow. Radiation exposure is often accompanied by several stressors. Although much is known about the effects of stress or radiation individually on the immune system, the combined effect of stress and radiation has not been thoroughly investigated. Therefore, to determine whether radiation synergizes with stress, mice were subjected to HU for 2 days and then exposed to 2 Gy gamma radiation. Mice were

euthanized after 6 hours or more of HU treatment, and apoptotic cells detected by DNA content analysis. HU-stressed mice had strikingly increased levels of apoptosis in the spleen and thymus compared to their unstressed counterparts, demonstrating that stress and radiation have a strongly synergistic effect on lymphocyte apoptosis. It must be emphasized that mice efficiently phagocytose cells at the early stages of apoptosis, such that the apoptotic cells appearing in our analyses are only those that have overwhelmed the phagocytic capacity *in vivo*. Further investigations on the role of Fas in this process *in vivo* are planned and it will be determined whether heavy particle radiation similarly synergizes with stress to cause lymphocyte apoptosis. 6) Because loss of the hematopoietic regenerative capacity of bone marrow cells has been associated with radiation exposure, and damage to hematopoietic cells is of major concern, the loss of hematopoietic potential in mice subjected to the same treatments by measuring the granulocyte-macrophage colony-forming ability of bone marrow has been assessed. Radiation exposure significantly reduced colony formation (Granulocyte-Macrophage (GM)-CFU), as previously reported. Stress by HU alone also moderately reduced the number of colonies. Importantly, the combination of stress and radiation synergistically reduced the number of colony forming units, causing a loss of 80%. In addition, the colonies from mice subjected to both radiation and HU were much smaller. Thus, the combination of stress and radiation not only enhanced lymphocyte apoptosis, but also synergistically impacted bone marrow colony formation.

5) Dr. Shearer. The hypothesis being addressed in Dr. Shearer's grant which has ended is that space flight radiation will suppress the human immune system leading to reactivation of latent viruses, increased viral infections and disease, and the development of cancers. A murine animal model of radiation and latent polyomavirus (PyV) infection was utilized to determine harmful effects upon the immune system with reactivation of latent virus infection. Key Findings of Project: Groups of BALB/c female mice were given whole body irradiation (3 Gy 137Cs) or sham irradiation on day 0 and 49, and murine polyomavirus (PyV) or saline control on day 1: A, 3 Gy + PyV; B, no Gy + PyV; C, 3 Gy + no PyV; and D, no Gy + no PyV. Mice were tested for PyV replication by quantitative PCR and spleen weights and cell counts, and proliferation and gamma interferon (IFN-g) production were measured at various intervals up to 69 days. Group A showed elevated PyV replication on days 10 and 20, as compared to Group B and both Groups A and B cleared PyV by day 49 in A and 20 in B. Only Group A again showed PyV replication when given a second dose of radiation on day 49. Spleen weights and cell counts of Group A were significantly lower than those of other groups. Irradiation suppressed T-cell proliferation in Groups A, B and C except in Group B when PyV was cleared. PyV infection enhanced IFN-g in all Groups: B > A > C. This model of space flight suggests that the combined effects of radiation and latent virus infection will severely affect T-lymphocyte mediated immunity that may lead to chronic viral infection and malignancy. Thus, these findings partially validate the hypotheses, complete the objectives, and reply to the specific aims of the original project. **Specific findings:** 1) Gamma radiation (3 Gy) delayed the clearance of polyomavirus and blunted gamma interferon production by spleen cells in response to virus infection in weanling mice. 2) The spleen weight, total cell count, and the lymphocyte proliferation were significantly reduced. 3) Radiation exposure produced virus reappearance (reactivation) in animals that had initially reduced virus infection to undetectable levels. 4) Only a small number of animals was followed for possible tumor formation; no tumors developed in the initial experiment observed for 300 days. **Conclusions:** These preliminary studies suggest that the combined effects of radiation and virus infection on the immune system of experimental animals lead to

immunosuppression and latent virus reactivation. Because of the striking effects upon immunocompetence and latent viral replication observed in these experiments, it is very likely that future studies will be able to offer surrogate marker tests for virus infection and immunocompromise, and new dietary and/or immunotherapeutic approaches to prevention and treatment of latent virus reactivation and lymphoid malignancies that develop as a consequence.

B) Publications

- 1) Aviles, H., Belay, T., Vance, M., Sun, B., and Sonnenfeld, G. Active Hexose Correlated Compound enhances the immune function of mice in the hindlimb-unloading model of space flight conditions. Revised and in final stages of review, *J. Appl. Physiol.*, 2004.
- 2) Aviles, H., and Sonnenfeld, G. Effects of Active Hexose Correlated Compound (AHCC) on resistance to infection of mice in the hindlimb-unloading model of space flight conditions. In: Kenner, D., editor. AHCC: Research and Commentary, Holodigm Publishers, In press, 2004.
- 3) Sonnenfeld, G. Volume Editor. *Experimentation with the animal model in space*, Volume in Advances in Space Biology, Elsevier, Amsterdam, In preparation for press, 2005.
- Aviles, H., Belay, T., Fountain, K., Vance, M. and Sonnenfeld, G. Increased susceptibility to *Pseudomonas aeruginosa* infection hindlimb unloading conditions. *J. Appl. Physiol.*, 95: 73-80, 2003.
- 4) Aviles, H., Belay, T., Fountain, K., Vance, M., Sun, B. and Sonnenfeld, G. Active hexose correlated compound enhances resistance to *Klebsiella pneumoniae* infection in mice in the hindlimb-unloading model of space flight conditions. *J. Appl. Physiol.* 95: 491-496, 2003.
- 5) Belay, T., Aviles, H., Vance, M., Fountain, K., and Sonnenfeld, G. Catecholamines and *in vitro* growth of pathogenic bacteria: Enhancement of growth varies greatly among bacterial species. *Life Sciences*, 73:1527-1535, 2003.
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C. Leveraging of Resources

The grant which ended in which Dr. Sonnenfeld was the PI produced a number of research findings which helped to support two new areas of research funded by NASA (representing a total dollar amount of \$1,357,387), and one non-Federal grant (from the Amino Up Chemical Company of Japan) (representing a total dollar amount of \$73,431).

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D. Space and Earth implications of the current accomplishments.

1) **Dr. Butel** The mouse model developed involving polyoma virus infection and radiation will allow targeted studies of the effects of irradiation on host immune function, virus infection, and tumor development. Such data will define the risk of these combined factors to long-duration space flight and will allow tests of countermeasures. It has been shown that

radiation can cause reactivation of latent viral infections and depress host immune function. The evidence for viral reactivations highlights the need to understand synergistic effects of radiation and space flight conditions that may result in more deleterious effects on immune function and control of microbial infections and disease. The knowledge gained from studies of virus reactivations in these test models will be applicable to earth-bound individuals at risk of suffering similar virus reactivations and serious, sometimes life-threatening, consequences due to immunosuppression following organ transplantation or cancer chemotherapy and during pregnancy, old age, and AIDS. Studies of the role of EBV latent genes in human disease may lead to the development of novel antiviral drugs.

2) Dr. Shearer (grant which ended during the past year) Humans undergoing multi-modal immunosuppression for organ or bone marrow stem cell transplantation occasionally (i.e., 2% occurrence) develop polyclonal activation of B cells due to the latent Epstein-Barr virus (EBV). Repetitive activation of B cells by EBV may lead to the rapid growth of B cell clones that undergo malignant transformation. The B cell lymphomas are now treated with two forms of immunotherapy—EBV-specific autologous T cell clones, and anti-B cell monoclonal antibody (Rituximab). These are treatments that could be quantitatively explored in the murine immunosuppressive model of radiation and latent virus infection. Use of this model would permit the animal trials of additional modes of immunotherapy, such as the use of designer fusion proteins containing tumor-specific antibody and B-cell toxic reagents. Thus, this space research on irradiated and virus-infected mice may yield valuable information for the treatment of lymphomas in humans. This research will also benefit humans on Earth because of the pioneering efforts to explore the intersection of immunodeficiency, virus infection, and cancer, such as seen in organ and bone marrow stem cell transplanted patients.

3) Dr. Sonnenfeld (grant which has ended during this past year). The development of AHCC as a potential countermeasure for immune dysfunction induced by space flight has led to a new grant from the Amino Up Chemical Company to study the use of AHCC to prevent infection during severe trauma.

4) Dr. Gewirtz. The development of effective radiation countermeasures could have significant impact on earth for civilians and military personnel alike. With regard to the civilian population, it is quite conceivable that results obtained will be relevant to patients undergoing cancer chemotherapy and radiation therapy. In this regard, it is possible that our studies will provide reagents and strategies for helping to protect normal tissues from the collateral damage of anticancer treatments. It is also possible that the results generated will be relevant to radiation workers, and members of the armed forces who may be exposed to radiation during the course of carrying out their respective duties.

IV. TEAM PLANS

The team composition has changed substantially since the last annual report, as described in the Abstract section. The first retreat as a newly organized team will occur on December 8, 2004 in Philadelphia – at the University of Pennsylvania. It is hoped that the retreat will encourage the team members to work together so that more synergies among the projects can be developed. Some of the individual team members shared their thoughts on the plans for the coming year, as described below.

Dr. Butel – 1) tissue specimens harvested from previous mouse polyoma virus space radiation experiments will be re-analyzed. The highly sensitive quantitative RQ-PCR assay will allow the recovery of additional data from those experiments and will lead to a better understanding of radiation effects on the host immune system. 2) Long-term animal

experiments will be undertaken in which mice infected with PyV and exposed to radiation will be observed for lymphoma and leukemia development. 3) We will measure virus reactivations in volunteers participating in the NASA Bed Rest Study Campaign 1, a model for the effects of space flight conditions on human physiology. 4) studies of radiation-induced signals that cause EBV reactivation from latency will be started. The necessary permits to import some needed engineered cell lines from London are being obtained. Conditions will be optimized to induce EBV replication and assays to quantitate reactivation in cell lines currently available. 5) It will be determined whether the effects of hind limb unloading (HLU) and irradiation synergistically affect control of polyoma virus infections (reactivation) and immune impairment. Flow cytometry will be used to determine whether virus infection, irradiation, and HLU of mice alters the distribution and numbers of individual leukocyte cell subsets compared to control mice. 6) The effect of irradiation and virus infection on cytokine expression will be determined by analyzing cytokine mRNA expression profiles in tissues collected from irradiated and nonirradiated polyoma virus-inoculated and control mice. mRNA levels of approximately 100 cytokines and chemokines by membrane arrays will be determined.

Dr. Shi 1) Since Fas plays a critical role in HU-induced splenocyte reduction and the origin of FasL is not known, it will be determined whether the effect of $CD4^+CD25^+$ regulatory T cells is due to their expression of FasL. $CD4^+CD25^+$ regulatory T cells will be purified from gld/gld (FasL mutant) mice and these cells will be transferred into SCID mice previously reconstituted with splenocytes depleted of $CD4^+CD25^+$ Treg cells. 2) The proteins identified from the serum of HU-treated mice will be characterized. In addition, it will be determined whether production of these proteins is related to endogenous opioids or glucocorticoids. 3) It will be determined whether HU-induced bone loss in the hind limbs is related to an over production of glucocorticoids by using RU486. 4) Mouse food developed as a countermeasure by Dr. Ann Kennedy will be utilized to determine whether dietary intervention can inhibit HU-induced and radiation induced lymphocyte reductions.

Dr. Gewirtz The main focus for the coming year will be to continue experiments characterizing the biological effect of exposure to the ground based-equivalents of cosmic radiation (heavy ions and protons). Drs. Gewirtz and Sutherland propose to combine their respective expertise in stem cell biology and DNA damage/repair to determine these effects. The studies to date suggest that human hematopoietic stem cells are exquisitely sensitive to even low doses of Fe^{26+} particles (see above). Accordingly, all studies proposed on the relative biological impact of HZE particles will be carried out in the dosage range deemed most relevant from our previous studies (<30 cGy). Additional particles (carbon, titanium, protons) have become available at the AGS (Brookhaven National Laboratories). Dose-response experiments using these new particles, as in the initial studies, will be carried out on the well-characterized populations of HSC and HPC and the functional consequences of such exposure using a variety of cell and molecular based assays will be analyzed. The molecular lesions responsible for cell damage will be further analyzed, which could be of considerable importance in identifying radioprotectants that might ameliorate, or prevent, the damages identified, an issue which will also be addressed. The planned experiments should detect, and quantify any immediate type of damage to various components of the blood cell generating system.

Muscle Alterations and Atrophy Team 2004 Report

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Project Title: Human Muscle Energetics and Mechanics

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I. Abstract.

The research mission of the Muscle Alterations and Atrophy Team (MAAT) is to ascertain the underlying mechanisms associated with the loss of muscle mass, strength, and endurance that are the cornerstones of the structural and functional deficits that occur when individuals (human and animal) are subjected to prolonged states of inactivity or skeletal muscle unloading. A key element of this research mission is to, over time, elucidate countermeasures that can effectively ameliorate these deficits using a variety of strategies such as exercise, nutritional and pharmacological interventions, as well as evolving the unique strategy of human powered artificial gravity (assuming that both NASA and the NSBRI becoming truly committed to exploring this modality as a countermeasure strategy). In the past fiscal year significant progress has been made in gaining a better understanding of 1) the factors that induce/affect muscle atrophy, 2) the key genes that are impacted by muscle unloading stimuli, 3) the functional consequences of the atrophy process, and 4) the efficacy of using a) relatively common therapeutic agents and b) a simple isometric resistance training program to significantly reduce the atrophy response in rodent skeletal muscle. This latter observation has the potential to be translated to human experimentation as a potential countermeasure. In the 2003/04 NSBRI NRA for the skeletal muscle system, a high priority for future research was centered on translational research involving humans in order to bring about a more balanced research portfolio bridging animal and human research. To that effort, a human project dealing with artificial gravity was recently accepted into the Muscle Team Research Portfolio. Thus the vision of the MAAT is certainly moving in the right direction

II. Introduction

In the Fall of 2000, the National Space Biomedical Research Institute's (NSBRI) Muscle Alterations and Atrophy Team (MAAT) began its second three-year funding cycle on research dealing with the structural and functional deficits of the skeletal muscle system that occur in response to prolonged exposure to space flight or the environment of microgravity. Of the eight original projects that were selected in the first period of funding (1997-2000), only one project was selected for continuation. The PI for the project was Dr. Alfred Goldberg, Harvard Medical School; and Dr Ken Baldwin, University of California, Irvine served as a co-investigator on that original project.

In the Fiscal-Year 2000-2003 funding cycle, seven new projects were selected for funding in addition to Dr. Goldberg's project. Two projects were selected for a funding cycle that started on October 1, 2000. These selections involved projects headed by Dr. Baldwin and Dr. Goldberg, respectively, who now serve as the Team Leader and Co-Team Leader for the MAAT. The remaining six projects were recently initiated in funding cycles at different starting dates in the 2001 calendar year. These projects are defined in the next section, Program Structure and Design. During Fiscal Year 2001-2002, two additional projects from the Integrated Function Team were reassigned to the MAAT such that there are now a total of ten research projects that define this team.

The research mission of the MAAT is to ascertain the underlying mechanisms associated with the loss of muscle mass, strength, and endurance that are the cornerstones of the structural and functional deficits that occur when individuals (human and animal) are subjected to prolonged states of inactivity or skeletal muscle unloading. A key element of this research mission is to, over time, elucidate countermeasures that can effectively ameliorate these deficits using a variety of strategies such as exercise, nutritional and pharmacological interventions, as well as evolving the unique strategy of human powered artificial gravity (assuming that both NASA and the NSBRI becoming truly committed to exploring the modality as a countermeasure strategy).

Muscle Deficits and Critical Concerns to Be Addressed by the Muscle Team.

The following deficits/concerns have been identified in the critical pathway roadmap of understanding astronaut health and safety during prolonged spaceflight. These include:

1. Reduced muscle mass (atrophy), which is thought to be due to an imbalance in protein synthetic to protein degradation activity within targeted fibers. The mechanism(s) for such a response is largely unknown.
2. Reduced muscle strength leading to a decrease in physical activity performance and high power output capacity. Deficits in strength often exceed the loss in muscle mass suggesting that more complex mechanisms are responsible for the reduced performance.
3. A slow-to-fast shift in the contractile protein phenotype, e.g., shifts to expression of faster myosin heavy chain (MHC) and calcium cycling proteins. These alterations induce the muscle fibers to become less economical in sustaining force output and locomotor activity.
4. A decreased resistance to fatigue, (which could have functional implications in the performance of extra vehicular activity in space and in performing emergency egress activity upon space craft landing.) This problem is relevant to the other deficits outlined above.
5. A proneness to muscle injury, which is due to the atrophy and loss of strength. An additional outcome of the muscle weakness could cause increased susceptibility to accidents that, in turn, could cause damage to other systems, e.g., bone fractures.
6. Changes in muscle properties are closely linked to changes in the ability of nervous system to accurately control movements; and thus such changes affect safety when performing any type of work.

III. Research Program Structure and Design

Listed below are the research topics and the associated Principal Investigators that form the backbone of the MAAT Strategic Plan. These include:

- Role of Muscle Loading Conditions on Mechanisms of Protein Translation and Their Impact on Unloading-Induced Atrophy -(PI: K. M. Baldwin; University of California, Irvine). This project addresses Concerns #1 and #2.
- The Activation of Protein Breakdown Upon Unloading and Possible Countermeasures—(PI: A.L. Goldberg; Harvard Medical School). This project addresses Concern #1.
- Calpains in Simulated Microgravity-induced Muscle Atrophy -(PI: P. B. Antin; University of Arizona). This project addresses Concern #1.
- Genomics of Human Skeletal Muscle During Bedrest and Exercise --(PI; M. Hamilton; University of Missouri, Columbia). This project addresses Concerns #1-4.
- Gene Expression Profiling of Unloaded Skeletal Muscle -(PI; S. Kandarian; Boston University). This project is also linked to Concerns #1-4
- In Vivo Stress Strain Dynamics in Human Muscle -(PI:S. Sinha; University of California, Los Angeles). This project addresses Concerns #4-6
- .Redux Modulation of Muscle Fatigue and Atrophy Processes in (Simulated) Microgravity -(PI; M. Reid; Baylor College of Medicine). This project addresses Concern # 2 and 4.
- Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle-(PI; P. B. Chase; Florida State University). This project addresses concerns #4-6.
- Calcium Homeostasis and Muscle Phenotype -(PI: R. Wiseman; Michigan State University). This project addresses Concerns #3 and 4.
- Human Muscle Energetics and Mechanics-(PI; M. Kushmerick; University of Washington). This projects addresses Concerns #4-6.

IV. Research Program Accomplishments

Key Findings From the Baldwin Team:

In years two and three which continued into FY 04, we focused on two specific project areas 1) characterizing the efficacy of different contraction modes for induce skeletal muscle hypertrophy, which could potentially evolve to a paradigm countermeasure for reducing unloading induced atrophy; and 2) assessing the effectiveness of short term isometric training regimens in blunting the rapid phase of unloading induced atrophy which occurs during the first week of unloading. The following are summaries as to what we accomplished in addressing these three important topics.

Skeletal Muscle Hypertrophy in Response to Isometric, Lengthening and Shortening Training Bouts of Equivalent Duration.

Movements generated by muscle contraction generally include periods of muscle shortening and lengthening as well as force development in the absence of external length changes (isometric). However, in the specific case of resistance exercise training, exercises are often intentionally designed to emphasize one of these modes. The purpose of the current study was to objectively evaluate the relative effectiveness of each training mode for inducing compensatory hypertrophy. Using a rat model with electrically stimulated contractions, groups of rats completed 10 training sessions in 20 days. Within each training session the stimulation duty cycle was equal across the three modes. While this protocol provided equivalent durations of duty cycle, the torque integral for the individual contractions varied markedly with training mode such that: lengthening > isometric > shortening. The results indicate that the hypertrophy response did not track the torque integral with mass increases of: isometric 14%, shortening 12% and lengthening 11%. All three modes of training resulted in similar increases in total muscle DNA and RNA. Muscle mass was highly correlated with the 10-session-mean force integral for isometric and shortening but not lengthening actions. The results of this study indicate that relatively pure movement mode exercises result in similar levels of compensatory hypertrophy that do not necessarily track with the total amount of force generated during each contraction. A paper has been published on this series of experiments (see below)

Isometric-Mode Exercise As a Countermeasure to Unloading Induced Atrophy

Based on the findings that isometric exercise appears to be as effective as both lengthening- and shortening contractions under high loading conditions. Therefore, we have initiated studies to examine the effectiveness of isometric contractions (4 sets of ten 2-second contractions with 20 second rest intervals between each contraction and 5 minutes of rest between sets) on its ability to reduce the early-onset of muscle atrophy that is a characteristic feature of the hindlimb suspension model. Preliminary findings clearly show that the mixed fibered medial gastroc (MG) muscle weight /normalized to body mass was significantly blunted relative to the contralateral muscle which was unloaded, but not resistance trained. These findings on rats suggest that the simple mode of isometric contraction can be effective in retarding the rapid loss of muscle weight that occurs during the early stages on unloading in which the muscles appear to be the most vulnerable to unloading induced muscle protein degradation. Additional findings show that the resistance loading paradigm inhibited the up regulation of two genes, e.g. atrogen and myostatin. These genes have been associated with playing a regulatory role in almost all muscle wasting models studied. In contrast, the resistance paradigm either maintained or augmented the expression of the growth factor IGF-1, which is known to control anabolic processes inducing muscle hypertrophy. Our findings suggest that programs that are successful in slowing down the rate of atrophy in the face of muscle unloading stimuli must do so by blunting the activation of genes associated with inducing a catabolic state while maintaining expression of genes known to create anabolic conditions to favor a positive protein balance in the muscle.

Resistance Exercise Involving Unloaded Human Skeletal Muscle

Previously it has been shown that the human ground based model consisting of unilateral limb suspension (ULLS) induces atrophy and reduced strength of the affected quadriceps muscle group. Resistance exercise (RE) involving concentric-eccentric actions, in the face of ULLS, is effective in ameliorating these deficits. The goal of the present study was to determine if alterations in contractile protein gene expression, e.g., myosin heavy chain (MHC) and actin, as studied at the pre-translational level, provide molecular markers concerning the deficits that occur in muscle mass/volume during ULLS, as well as its maintenance in response to ULLS plus RE. Muscle biopsies were obtained from m. vastus lateralis of 31 middle-aged men and women before and after five weeks of ULLS, ULLS plus RE or RE only. The RE paradigm comprised 12 sessions of four sets of seven concentric-eccentric knee extensions. Our findings show that there were net deficits in total RNA, total mRNA, and actin and MHC mRNA levels of expression following ULLS ($P < 0.05$); whereas, these alterations were blunted in the two groups receiving RE. Additional observations involving IGF-I and its associated receptor and binding proteins suggest that RE postures the skeletal muscle for signaling processes favoring a greater anabolic state relative to that observed in the ULLS group. Collectively these findings suggest that molecular markers of contractile protein gene expression serve as useful subcellular indicators for ascertaining the underlying mechanisms regulating alterations in muscle mass in human subjects in response to altered loading states. A paper has been published on this experiment.

Publications

Adams, G. R., D. C. Cheng, F. Haddad and K. M. Baldwin. Skeletal muscle hypertrophy in response to isometric, lengthening and shortening training bouts of equivalent duration. *J. Appl. Physiol.* 96: 1613-1618, 2004

Haddad, F., K. Baldwin, and P.A. Tesch. Pretranslational markers of contractile protein expression in human skeletal muscle: effects of limb unloading plus resistance exercise. *J. Appl. Physiol.* 97:000-000, published on line Sept, 04

Key Findings From the Goldberg Team.

Significant Findings in 2003-2004

1) Skeletal muscle atrophy is a debilitating response to weightlessness and disuse, as well as fasting and many systemic diseases including diabetes, cancer, and renal failure. We have identified a common set of about 50 genes (termed atrogenes) that are strongly induced or suppressed in muscles in these diverse catabolic states.

2) Among the strongly induced genes were many involved in protein degradation, including polyubiquitins, the Ub ligases atrogin-1/MAFbx and MuRF-1, multiple (but not all) subunits of the 26S proteasome. Among those downregulated are genes required for ATP production, for extracellular matrix proteins, and for several growth-related mRNAs (P311, JUN).

3) Using in vitro models of atrophy in cultured muscle cells, we showed that the catabolic hormone dexamethasone inhibits growth and enhances the breakdown of myofibrillar proteins, while also increasing atrogen-1 and MuRF1 mRNA. By contrast, the growth factors IGF-1 and insulin prevented these catabolic responses.

4) IGF-1 rapidly reduces atrogen-1 mRNA by blocking its transcription and that of other important atrophy-related genes (atrogenes).

5) IGF-1 and insulin activate the PI3-kinase/Akt pathway, which suppresses proteolysis and atrogen-1 mRNA expression in muscle.

6) We also showed that in in vitro models of atrophy, there is a decrease in the PI3K/AKT pathway, activation of the forkhead (Foxo) family, and induction of atrogen-1.

7) Furthermore, IGF-1 by activating AKT causes Foxo inhibition, and thus blocks atrogen-1 expression.

8) Foxo3, by acting directly on the atrogen-1 promoter, causes atrogen-1 expression and by itself causes dramatic atrophy of cultured myotubes and fibers in adult mouse muscles.

9) When Foxo activation is blocked (by a dominant negative construct or RNAi), the induction of atrogen-1 by glucocorticoids and the resulting reduction in myotube size are prevented. Thus, inhibition of Foxo function could be a novel approach to combat various forms of muscle wasting.

3) Technical Breakthroughs and Pending Patents

Patents pending:

A patent has been applied for based on our demonstration of the critical role of the Foxo family of transcription factors in atrophy. These findings indicate that Foxo and the PI3 signal transduction system are attractive targets for drug development to combat muscle wasting.

Goldberg, AL, Sandri, M, and Lecker, SH. Foxo transcription factors are novel pharmacological targets to inhibit skeletal muscle atrophy. Submitted US Patent Office, Dec 2003.

Technical breakthroughs:

1) We have further defined a set of genes whose level changes markedly whenever a muscle atrophies (independently of the physiological signal, i.e., with disuse, nerve injury, fasting, diabetes, cancer, renal failure, elevated glucocorticoids).

2) We have shown that an important rapid action of growth-promoting agents and contractile work as to suppress expression of the atrophy-related ubiquitin-ligase, atrogin-1.

3) We have identified the signaling pathway by which growth factors IGF-1 and insulin suppress the expression of atrophy-related transcriptional response.

4) We have shown that the transcription factor, Foxo3, triggers the expression of atrogin-1 during atrophy, and that growth factors lead to its phosphorylation and inactivation.

4) Collaborations within the team, NSBRI, and outside NSBRI

Dr. Kenneth Baldwin, Dept. of Physiology and Biophysics, Univ. California at Irvine

Dr. Reggie Edgerton, UCLA

Dr. Stefano Schiaffino, University of Padua, Padua, Italy

Dr. Stewart Lecker, Dept. Medicine, Beth Israel Medical Center, Boston, MA and Harvard Medical School

5) Invited Presentations

Sandri, M, Sandri, C, Gilbert, A, Schiaffino, S, Lecker, S, and Goldberg, AL. "Foxo Transcription Factors Induce the Atrophy-Related Ubiquitin Ligase, Atrogin-1, and Cause Skeletal Muscle Atrophy." 2004 Annual NSBRI Workshop, Lake Conroe, TX

Goldberg, AL. "Induction of Atrophy-Related Genes and the Ubiquitin Ligase, Atrogin-1, in Muscle during Disease States." 2004 Symposium on Protein Ubiquitylation in Health and Disease, Weizmann Institute of Science, Israel

Goldberg, AL. "Mechanisms for Induction of the Key Ubiquitin Ligase, Atrogin-1, and Muscle Atrophy in Disease States." 2004 Ubiquitin Drug Discovery Summit, Philadelphia

Goldberg, AL. "Regulation of the Expression of Atrophy-Specific Ubiquitin-Ligases, Atrogin-1 and MuRF1, in Normal and Atrophying Muscle." 2004 Mouchly Small Muscle Symposium, "Mechanisms of Muscle Atrophy," Amherst, MA

Goldberg, AL. "Functions of the Proteasome in Cell Regulation and Immune Surveillance: From Basic Understanding to Human Therapy." 2004 Annual American Society of Nephrology meeting, St. Louis, MO

6) Publications from September 2003-October 2004

Li, Y, Lecker, SH, Chen, Y, Waddell, ID, Goldberg, AL, and Reid, MB. TNF- α stimulates ubiquitin conjugation in skeletal muscle by upregulating UbcH2/E2_{20K1}. FASEB J 2003; 17:1048-1057.

Lecker, SH, Jagoe, RT, Gilbert, A, Gomes, M, Baracos, V, Bailey, J, Price, SR, Mitch, WE, and Goldberg, AL. Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. FASEB J 2004; 18: 39-51.

Sandri, M, Sandri, C, Gilbert, A, Skurk, C, Calabria, E, Picard, A, Walsh, K, Schiaffino, S, Lecker, SH and Goldberg, AL. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. Cell 2004; 117: 399-412.

Jagoe, RT, Tawa, Jr., NE, and Goldberg, AL. Protein and amino acid metabolism in muscle. In: Engel, A, and Franzini-Armstrong, C (editors). *Myology*, vol. 1, 3rd ed. New York: McGraw-Hill, 2004. p. 535-564.

Sacheck, JM, Ohtsuka, A, McLary, SC and Goldberg, AL. IGF-1 stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1. Am J Physiol Endocrinol 2004; 287: E591 - 601.

Key Findings From the Antin Team:

No report was submitted. The antin project was terminated in May, 2004

Key Finding From the Sinha Team.

▪ Significant findings/ Accomplishments

1. A non-invasive method combining MR imaging techniques with MR-compatible dynamometry was developed and utilized to quantify the effect of atrophy in the human lower leg. The measured variables include volume of different muscle compartments, ankle joint torque and strain properties of the ankle plantarflexor muscles and tendinous structures of the muscles. In this year, we had the opportunity to develop this technique on a 3T MRI scanner, which gives a distinct advantage of signal-to-noise over the previously used 1.5T machine.
2. For 5 subjects, atrophy was induced using a 4-week unilateral limb suspension (ULLS) model followed by 6-week rehabilitation, previously validated the previous two years. The ULLS model used was very effective for inducing localized atrophy, completely reversible and well-tolerated by the normal volunteers. The measurement method was accurate and reliable in detecting changes in the above-mentioned parameters before and after ULLS and the return to normalcy throughout the prescribed rehabilitation.
3. Preliminary results of the previous ULLS study (performed in the prior years of the grant) showed that the decrease in muscle volumes following ULLS was

proportionate to similar changes in relative strain distribution of the tendinous structures, suggesting that the structure and function are tightly coupled.

4. Preliminary results of the most recent ULLS study (performed in 2004) demonstrated that absolute strain distribution (assessed with 10 and 20 % Pre-ULLS MVC) of tendinous structures changed following ULLS, possibly due to different motor unit recruitment strategy and altered mechanical properties of the structures. In addition, a different rehabilitation paradigm with moderate resistance training protocol (using loading condition less than subject's body weight) was tried out and found to be very effective.

▪ **Technical breakthrough and Patent**

Development of a device compatible with a 3 Tesla MR scanner, with which the subject can exert plantarflexion motion under different loading conditions, while MR scans were acquired to measure velocity and strain in different parts of the lower leg anatomy at different time points of the force cycle. No patent has been applied for as of now.

II. Implications for the welfare of Astronauts in Space Environment:

One of the most significant problems facing astronauts is that of muscle atrophy resulting from micro-gravity or immobilization during space flights. Methods that can reveal the functional status of different muscle groups, in a robust, clinical and preferably non-invasive and safe manner, could have a significant clinical impact in the physical well being of the astronaut. Knowledge about how the strain distribution, muscle volume and the maximum force the subject can exert, change as atrophied muscle progresses towards normalcy can be a valuable clinical parameter to quantify the relative efficacy of different therapeutic strategies for rehabilitation used for astronauts, and can help one understand the complex interactions between active contractile tissues and passive connective ones. The method we have developed, using phase contrast, velocity encoded MR imaging technique is such a non-invasive, non-ionizing and safe method that can be used routinely in the clinic. We have been able to prove its efficacy in monitoring the changes after onset of atrophy and the method is sensitive enough to follow the changes as the muscles return to normalcy as the patient is given rehabilitative physio-therapy.

▪ **Collaborations within the team, NSBRI, and Outside NSBRI**

None.

▪ **Presentations at Science meetings, workshop, using NSBRI research data**

Papers (Published)

1. Sinha, S., Hodgson, J. A., Finni, T., Lai, A. M., Grinstead, J., Edgerton, V. R. "Muscle Kinematics During Isometric Contraction: Development of Phase Contrast and Spin Tag Techniques to Study Normal and Atrophied Muscles." Accepted for Publication in J Mag. Res Imag in December, 2004.
2. Ph.D. Thesis, "MRI of In Vivo Human Triceps Surae Motion During Contraction After Atrophy and Achilles Tendon Rupture", by Alexander Lai. UCLA, Dept. of Molecular, Cellular, and Integrated Physiology, Nov. 2002.

Papers (Submitted):

3. Finni, T., Hodgson, J.A., Lai, A., Edgerton, V.R. and Sinha, S. "Muscle Synergism during Isometric Plantarflexion in Achilles Tendon Rupture Patients and in Normal Subjects revealed by Velocity-Encoded Cine Phase-Contrast MR". Submitted to Radiology, Sept. 2004.

Papers (From Previous two years of the NSBRI Grant):

4. Finni T, Hodgson JA, Lai AM, Edgerton VR, Sinha S. Nonuniform strain of human soleus aponeurosis-tendon complex during submaximal voluntary contractions in vivo. J Appl Physiol. 2003 Aug;95(2):829-37.
5. Finni T, Hodgson JA, Lai AM, Edgerton VR, Sinha S. Mapping of movement in the isometrically contracting human soleus muscle reveals details of its structural and functional complexity. J Appl Physiol. 95:2128-2133, 2003.

Refereed Conference Publications

1. Hodgson, J. A., Roiz, R., Finni, T., Lee, H. D., Edgerton, V. R., and Sinha, S. Amplification of muscle fiber length changes in human soleus muscle-tendon complex. The 28th Annual Meeting of American Society of Biomechanics, Portland, USA Sept. 8-11(2004).
2. Lee, H. D., Hodgson, J.A., Finni, T., Edgerton, V.R., and Sinha, S. Strain distribution of in vivo human triceps surae during passive and active dynamic movements. The 28th Annual Meeting of American Society of Biomechanics, Portland, USA Sept. 8-11 (2004).
3. Sinha, S., Finni, T., Hodgson, J.A., Lai, A.M., Edgerton, V.R. "MR Phase Contrast Study of Differences in StructureFunction Relationship during Isometric and Passive Movement of Lower Leg." The12th Intl. Soc Mag Res Med Mtng. Kyoto, Japan (2004).
4. Sinha, S., Lee, H. D., Finni, T., Hodgson, J.A., Edgerton, V.R. "An MR phase-contrast in-vivo study of Strain Distributions during Passive and Dynamic Movements of the Muscles of the Lower Leg". Accepted for presentation at the 92nd Mtng. of RSNA, Chicago, USA (2204).
5. Sinha, S., Lai, A.M., Hodgson, J.A., Finni, T., Grinstead J and Edgerton, V.R. Functional MR Characterization of Atrophied Human Triceps Surae Muscle following Four weeks of Lower Limb Suspension. The 91st Mtng of RSNA, Chicago, USA (2003).
6. Sinha, S., Finni, T., Hodgson, J.A., and Edgerton, V.R. Structure-Function Correlation of the Human Soleus Muscle. The 91st Mtng of RSNA, Chicago, USA (2003).
7. Sinha, S., Lai, A.M., Finni, T., Hodgson, J.A., Edgerton, V.R. Assessment of Functional Recovery after Surgical Repair of Achilles Tendon Rupture from Estimates of Contraction Velocity, Muscle Volume and MVC". The 91st Mtng of RSNA, Chicago, USA (2003).

Key Findings From the Kandarian Team.

Significant findings in the FY (September 2003 to October 2004).

- Nedd4, a HECT domain containing E3 ubiquitin ligase, was increased over a time course of unloading at both mRNA and protein levels. Nedd4 was found to be localized to the subsarcolemma of muscle cells, consistent with the location of its known substrates.
- Overexpression of Nedd4 in soleus muscles using electrotransfer of a Nedd4 expression plasmid led to increased ubiquitination of protein from whole muscle homogenates, but it did not affect muscle fiber cross-sectional area
- Microarray analysis was performed on mature myotubes in “starved” (non-fed) vs control conditions. A marked change in phenotype was found in the atrophied myotubes but it was quite different from that described in papers on whole muscle atrophy due to unloading, starvation, or cachexia.
- Adenoviral mediated expression of Atrogin-1 in myotubes was preferentially localized to the nucleus in “starved” myotubes but not fed or hypertrophied myotubes.
- Although muscle atrophy is associated with oxidative stress, adenoviral overexpression of catalase did not ameliorate markers of atrophy in starved myotubes or in TNF treated myotubes.
- Disruption of microfilaments or microtubules in cultured myotubes did not activate an in vivo marker of unloading atrophy (i.e., NF- κ B activity).
- TNF-induced activation of NF- κ B in myotubes was inhibited by disruption of microfilaments or microtubules

Technical breakthroughs and pending patents

- Wild-type and dominant negative Nedd4 adenoviral expression vectors have been constructed in order to study the role of Nedd4 on markers of atrophy in cultured muscle cells
- Adenoviral expression vectors of several additional proteolytic genes have been constructed including atrogin (cDNA from Dr. Goldberg), calpastatin (cDNA from Dr. Antin), and cathepsin C so they may also be studied for both individual and combined roles on markers of muscle atrophy in myotubes.
- A differentiated muscle (muscle creatine kinase (MCK)) promoter has been used to construct adenoviral vectors for improved specific expression of transgenes in muscle cell culture.

Presentations at Science Meetings, Workshops using NSBRI research data. (Don't list seminars at other institutions)

Presentation of research at the S. Mouchly Small Muscle Symposium – Mechanisms of muscle atrophy. Title of talk, “Intracellular signals in disuse muscle atrophy.” Host: Priscilla Clarkson, University of Massachusetts Amherst, June 24-25, 2004.

Presentation of research at the NSBRI Investigator Retreat, "Production of Adenoviral Expression Vectors to Study the Role of Proteolytic Genes in Muscle Atrophy." Del Lago Conference Center, Montgomery, TX, Jan 12-14, 2004

Publications/Abstracts in the designated FY.

Publications

Jackman, R.W. and S.C. Kandarian. The Molecular Basis of Skeletal muscle atrophy. *Am J. Physiol. Cell Physiol.* 287: C834-C843, 2004.

Stevenson, E.J. and S.C. Kandarian. Gene expression profiling shows its muscle. *Physiology News.* 54: 24-25, 2004.

Stevenson, E.J., P.G. Giresi, A. Koncarevic, and S.C. Kandarian. Global analysis of expression patterns during disuse atrophy in rat skeletal muscle. *J. Physiol.* 551.1;33-48, 2003.

Key Findings From the Bryant Team.

Significant findings:

- Nano-mechanical model of the muscle sarcomere (a component of the Digital Astronaut):
 - Presence of filament compliance could modulate the dependence of steady state isometric force and the rate of tension development on thin filament activation level.
 - Tuning of biomechanics at the protein level may be as significant as at higher levels of organization and needs to be considered in biomechanical components of physiological models to describe changes in muscle associated with exercise and microgravity.
- Signaling pathways in muscle plasticity:
 - The immunosuppressant drug rapamycin has little or no effect on maximum Ca^{2+} -activated force, but (at some concentrations) increases Ca^{2+} -sensitivity of isometric force and inhibits the kinetics of tension redevelopment; this suggests that rapamycin affects Ca^{2+} -regulatory proteins (troponin/tropomyosin) more than actomyosin.
 - Attenuation of cardiac hypertrophy by rapamycin is not due to direct effects of rapamycin on myofilament contractility

Patents:

- none filed under NSBRI funding

Collaborations:

- Michael Regnier, Dept. of Bioengineering, University of Washington (Co-Investigator)
- Robert W. Wiseman, Depts. of Radiology and Physiology, Michigan State University
- Martin J. Kushmerick, Depts. of Radiology and Physiology & Biophysics, University of Washington

Presentations:

- 2003 - "Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle," Bioastronautics Investigators' Workshop, Galveston, TX (oral)
- Fredlund J, Regnier M and PB Chase. 2003. Inorganic phosphate (Pi) dependence of force in fast and slow fibers from different rat and rabbit skeletal muscles. *Biophys J.* 84:450a. (poster at Biophysical Society meeting)
- 2004 - "Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle," NSBRI Investigators Meeting, Houston, TX (poster)
- "Thermodynamics of the Creatine Kinase Reaction: Effects of varying pH, pMg and Temperature," R.W. Wiseman, C. Brown, J Brault, T.W. Beck, A.M. Gordon and P.B. Chase, NSBRI Investigators Meeting, Houston, TX (poster)
- "Cell & Molecular Biomechanics: Dynamics of Ca^{2+} Regulation and Muscle Performance" Ohio State University's Mathematical Biology Institute Workshop on Signal Transduction II: Muscles and Synapse, Columbus, OH (oral)
- Kataoka A, Schoffstall B, Clark A and PB Chase. 2004. Effect of rapamycin on skinned skeletal muscle fiber force. *Biophys J.* 86:213a. (poster at Biophysical Society meeting)
- Tanner BCW, Regnier M, Chase PB and TL Daniel. 2004. Modeling different nearest-neighbor interactions with a spatially explicit, Calcium-regulated, compliant myofilament model. *Biophys. J.* 86:566a. (poster at Biophysical Society meeting)
- "Effects of rapamycin on cardiac and skeletal muscle contraction and crossbridge cycling," B. Schoffstall, A. Kataoka, A. Clark and P.B. Chase. American Heart Association Council on Basic Cardiovascular Sciences: Stress Signals, Molecular Targets and the Genome, Stevenson, WA (poster)
- "Spatially Explicit, Nano-Mechanical Models of the Muscle Half-Sarcomere: Implications for Biomechanical Tuning in Atrophy and Fatigue," P.B. Chase, A. Kataoka, B.C.W. Tanner, J.M. Macpherson, X. Xu, Q. Wang, M. Regnier and T.L. Daniel. International Astronautical Congress, Vancouver, BC, Canada (oral)
- 2005 - (future presentation) "Cell and Molecular Biomechanics: Cardiac and Skeletal Muscle," Bioastronautics Investigators' Workshop, Galveston, TX (poster)
- (future presentation) Hemmer C, Kataoka A and P B Chase. 2005. Mathematical simulation of the effects of familial hypertrophic cardiomyopathy mutations on Ca^{2+} transients and force generation," *Biophys. J.* 88: In press. (poster at Biophysical Society meeting)

Publications:

1. Chase, P.B., J.M. Macpherson, and T.L. Daniel. 2004. A spatially explicit nano-mechanical model of the half-sarcomere: myofilament compliance affects Ca^{2+} -activation. *Ann. Biomed. Eng.* 32:1556-1565.
2. Schoffstall, B., A. Kataoka, A. Clark, and P.B. Chase. 2004. Effects of rapamycin on cardiac and skeletal muscle contraction and crossbridge cycling. *J. Pharmacol. Exp. Ther.* In press (published online 11 August 2004).

Published Abstracts:

1. Fredlund, J., M. Regnier, and P.B. Chase. 2003. Inorganic phosphate (Pi) dependence of force in fast and slow fibers from different rat and rabbit skeletal muscles. *Biophys. J.* 84:450a.
2. Hemmer, C., A. Kataoka, and P.B. Chase. 2005. Mathematical simulation of the effects of familial hypertrophic cardiomyopathy mutations on Ca^{2+} transients and force generation. *Biophys. J.* 88: In press.
3. Kataoka, A., B. Schoffstall, A. Clark, and P.B. Chase. 2004. Effect of rapamycin on skinned skeletal muscle fiber force. *Biophys. J.* 86:213a.
4. Tanner, B.C.W., M. Regnier, P.B. Chase, and T.L. Daniel. 2004. Modeling different nearest-neighbor interactions with a spatially explicit, Calcium-regulated, compliant myofilament model. *Biophys. J.* 86:566a.

Key Findings From the Reid Team.

- Oxidant activity is increased in soleus muscles 48 hrs after unloading and remains elevated for up to 12 days. (Hindlimb-suspended mice)
- Hydrogen peroxide stimulates ubiquitin conjugating activity and upregulates E3 proteins, including atrogin1/MAFbx and MuRF1. (C2C12 myotubes)
- Loss of specific force in unloaded soleus can be significantly inhibited by oral administration of allopurinol, a xanthine oxidase inhibitor and nonspecific antioxidant. (Hindlimb-suspended mice)
- Fatiguing exercise inhibits activity of nuclear factor- κB (NF- κB), a procatabolic transcription factor. (isolated muscles, hindlimb-suspended mice, humans)
- Onset of handgrip fatigue (as occurs in EVA) can be delayed by oral administration of N-acetylcysteine. (Human studies)

Technical advances:

- Worked with collaborators at NASA/JSC and Lockheed-Martin to develop handgrip ergometer for use on International Space Station. (Human studies)

Collaborations:

- Fred Goldberg, Harvard (muscle team)
- Ann Kennedy, University of Pennsylvania (NSBRI Team Leader; Immunology, Infection and Hematology)
- Scott Smith, NASA JSC, Houston (nutritionist; Human Adaptation and Countermeasures Office)
- Jeffrey Jones, NASA/JSC, Houston (Shuttle/ISS flight surgeon)
- Samme Landsdowne, Lockheed-Martin, Houston (ergometry software specialist)

Presentations

“Can Antioxidants Improve Muscle Performance?” USA Track and Field Coaches Workshop. Las Vegas, Dec. 2003.

“Redox Modulation of Muscle Function in Microgravity,” NSBRI Investigators’ Retreat, Del Lago Resort, Jan. 2004.

“Regulation of Muscle Adaptation by Redox Signaling,” Experimental Biology ’04, Orlando FL, May, 2004.

“Exercise Regulation of the Ubiquitin-Proteasome System,” Experimental Biology ’04, Orlando, FL, May 2004.

Publications

Li, Y.-P., Y. Chen, A.S. Li, and M.B. Reid. Hydrogen peroxide stimulates ubiquitin conjugating activity and expression of genes for specific E2 and E3 proteins in skeletal muscle myotubes. *Am J Physiol* 285:C806-C812, 2003.

Razeghi, P., S. Sharma, J. Ying, S. Stepkowski, M.B. Reid, H. Taegtmeier. Atrophic remodeling of the heart in vivo simultaneously activates pathways of protein synthesis and degradation. *Circulation* 108:2536-2541, 2003.

Matuszscak, Y., Arbogast, S., Reid, M.B. Allopurinol mitigates muscle contractile dysfunction caused by hindlimb unloading in mice. *Aviat Space Environ Med* 75:581-588, 2004.

Durham, W.J., Li, Y.-P., Gerken, E., Farid, M., Arbogast, S., Wolfe, R.R., Reid, M.B. Fatiguing exercise reduces DNA-binding activity of NF- κ B in skeletal muscle nuclei. *J Appl Physiol* 2004. In press.

Arbogast,S., Reid, M.B. Oxidant activity in skeletal muscle fibers is influenced by temperature, CO₂ level, and muscle-derived nitric oxide. *Am J Physiol Regul Integr Comp Physiol* 2004. In press.

Reid, M.B. Effects of exercise and decreased muscle use on the ubiquitin-proteasome pathway. *Am J Physiol Regul Integr Comp Physiol* In review.

Matuszcak,Y., Ferid,M., Jones,J., Lansdowne,S., Taylor,A., Reid, M.B. N-acetylcysteine inhibits muscle fatigue and glutathione oxidation during handgrip exercise. *Muscle & Nerve* In review.

Key Findings From the Hamilton Team.:

Summary of Recent Accomplishments:

- A translational approach of complementary and comparative studies in humans and rodents is being performed to identify novel genes and cellular processes that cause deleterious muscle alterations during microgravity or physical inactivity on earth.
- Transcriptional responses that could act as the initial triggers causing muscle alterations were identified in both human and rat studies.
- We reported the set of genes most sensitive to unloading and reloading in the classical rat model of unloading and reloading.
- Novel skeletal muscle factors involved in the regulation of transcription, protein turnover, cell signaling, and lipid or glucose metabolism were identified.
- Expression of some of the novel changes in gene expression have been confirmed and linked to cellular responses of skeletal muscle. One such transcriptionally-dependent process was up-regulated during unloading (in muscle from both humans and rats after unloading) and was experimentally shown in rats to be essential for a profound loss of muscle LPL protein and activity.

New or Recent Collaborations:

- Gender comparisons during unloading and reloading skeletal are being performed with Dr. M. Brown (Gender Differences in Skeletal Muscle, Bone and Articular Cartilage during unloading).
- We are collaborating with Dr. S. Powers in studies of ventilatory muscles during unloading.
- Collaboration with Dr. B. Ruby was performed in skeletal muscle studies associated with unloading/exercise paradigms in humans.

National meetings where NSBRI research was presented:

NSBRI conference (Montgomery Tx).
ACSM National Meeting

Publications During Last Fiscal Year:

Bey L, Akunuri N, Hoffman PE, Zhao P, Hamilton DG, Hamilton MT. Patterns in global gene expression in rat skeletal muscle during unloading and low-intensity ambulatory activity. *Physiol Genomics*;13:157-167, 2003.

Bey L, Hamilton MT. A molecular reason to maintain daily low-intensity activity: suppression of skeletal muscle lipoprotein lipase activity during physical inactivity. *J Physiol (London)*;551(2):673-682, 2003.

Hamilton MT, Bey L, Zderic TW, and Hamilton DG. Identification of the human physical inactivity and unloading genome. MSSE (Abstract Supplement), 2004.

Key Findings From the Wiseman Team.

Novel findings:

p38 MAP kinase is a signal transduction enzyme purported to be involved in mechanosensory activation of muscle gene expression. P38 MAP kinase is thus a molecular target for pharmaceutical research because of its role in muscle but also cardiovascular remodeling. These results bear on potential countermeasures of exercise as well as a new view of the mechanism of activation of this cascade in muscle.

- A novel inhibitor of cross-bridge formation reduces force with no loss in Ca^{2+} cycling in isolated fast and slow muscles.
- Contraction associated phosphorylation of p38 MAP kinase is not blocked when force is inhibited. Thus, p38 MAP kinase can not be a mechanosensor as proposed but may respond to other aspects of contraction.

Energetic demands limit muscle performance and may also modulate muscle phenotype. The limit to performance has long been thought to be calcium handling by the sarcoplasmic reticulum. This is an energetically fueled pump that is believed to stop functioning when the ATP free energy is approximately -50 KJ/Mol. Recent work in collaboration with investigators at Michigan State University, University of Nijmegen and the University of Missouri have shown that transgenic animals deficient in adenylate kinase function normally even at potentials as low as -45 kJ/Mol. This suggests significant adaptive changes in these animals and forces reinvestigation of the energetics of calcium handling.

- Adenylate kinase deficiencies lead to huge energetic challenges that are overcome by as yet to be understood changes in muscle design.

In collaboration with Drs Weiringa and Terjung, AK KO mice were studied by magnetic resonance spectroscopy. Cellular energetic changes that occur in these muscles are far greater

Publications: (Status-funding)

Ontiveros, C, R Irwin, RW Wiseman and LR McCabe. 2004. Hypoxia suppresses runx2 independent of modeled microgravity. Journal of Bone and Mineral Research (in print-NASA, NSBRI).

RC Burrows, SD Freeman, RW Wiseman, KA Krohn, M Muzi and AM Spence. 2004. ¹⁸FDG transport kinetics in the rat 36B-10 glioma and normal human fibroblast cell lines. Nuc. Med. Biol. 1:1-9. (NIH)

Accepted:

Meyer, RA, TF Towse, RW Reid, RC Jayaraman, RW Wiseman, and KK McCully. 2003. BOLD MRI mapping of transient hyperemia in skeletal muscle after single contractions. NMR in Biomedicine. (accepted with revisions-NIH, NSBRI)

Meyer, RA and RW Wiseman. 2003. The metabolic systems: Control of energy metabolism in skeletal muscle. In Exercise Physiology. Ed. C. Tipton. Elsevier. (accepted- NIH, NSBRI).

Presentations at National Meetings (form- funding)

Dentel, JN, SG Blanchard, DP Ankrapp, LR McCabe and RW Wiseman. 2004. The force of contraction is not responsible for mitogen activated protein kinase phosphorylation in mouse fast-twitch skeletal muscle during exercise. The Physiologist, 47:319. **(Poster- NSBRI).**

Brault, JJ, CR Hancock, RL Terjung, RA Meyer and RW Wiseman, 2004. Phosphate metabolites and pH in muscle of AK1 knockout mice during repeated bouts of intense contraction. The Physiologist, 47:334. **(Poster-NIH/NSBRI).**

Slade, JM, TF Towse, JJ Brault, RW Wiseman, MC Delano and RA Meyer. 2004. A gated 31P-NMR protocol for measurement of contractile ATP cost and PCr recovery without intense exercise. The Physiologist, 47: 334. **(Poster-NIH, NSBRI).**

Towse, TF, JJ Brault, **RW Wiseman**, RA Meyer. 2004. Functional MRI of motor cortex activation during fatiguing isometric handgrip contractions. American College of Sports Medicine 51st Annual Meeting, Indianapolis, IN. **(poster-NIH, NSBRI).**

Jayaraman, RC, DP Ankrapp, JN Dental, **RW Wiseman**. 2004. *In Situ* Electrical Stimulation on Early Acute mRNA Expression in Fast-Twitch Mouse Muscle. American College of Sports Medicine 51st Annual Meeting. Indianapolis, IN. **(poster-NSBRI).**

Latourette, MT, RC Jayaraman, **RW Wiseman**. 2004. A rapid and unbiased algorithm to quantify skeletal muscle twitch kinetics. American College of Sports Medicine 51st Annual Meeting. Indianapolis IN. **(poster-NSBRI).**

Ankrapp, DP, WA LaFramboise, KL Bombach, RC Jayaraman, and **RW Wiseman**. 2004. Global gene expression changes in mouse fast twitch muscle in response to electrical stimulation. 2004. Experimental Biology, Annual Meeting Washington DC. **(poster-NSBRI)**.

Dentel, JN, DP Ankrapp, SG Blanchard, AS Trudell, **RW Wiseman**. 2004. Mechanical strain is not the mechanism for p38 mitogen-activated protein kinase (MAPK) phosphorylation in fast skeletal muscle. Experimental Biology Annual Meeting, Washington, D.C. **(poster-NSBRI)**.

Bieber, BA, H Cirrito, M Heydens, C Whitehead, and **RW Wiseman**. 2004. Cellular energetics of INS-1 cells in response to a glucose challenge. Experimental Biology Meeting, Washington, DC. **(poster-NIH)**.

Wiseman, RW, C Brown, JJ Brault, TW Beck, AM Gordon and P. Bryant Chase. 2004. Thermodynamics of the Creatine Kinase Reaction: Effects of varying pH, pMg and Temperature. National Space Biomedical Institute Annual Meeting, Del Lago, Texas. **(Poster - NIH,NSBRI)**.

Ankrapp, DP, WA LaFramboise, KL Bombach, RC Jayaraman, and **RW Wiseman**. 2004. Altered Gene Expression of Mouse Fast-Twitch Skeletal Muscle in Response to Electrically Induced Contractions: Effects of Activity and Ryanodine Receptor Activation. National Space Biomedical Institute Annual Meeting, Del Lago, Texas. **(Poster- NSBRI)**.

Ontiveros C, R Irwin, **RW Wiseman** and LR McCabe. 2003. Hypoxic Conditions of the RWV decreases RUNX2 and osteoblasts differentiation. J Bone Min Res. 18: S343. **(poster-NASA, NSBRI)**.

DP Ankrapp, RC Jayaraman, JN Dentel, ER Wehrwein and **RW Wiseman**. 2003. The effects of electrical stimulation and caffeine administration on slow muscle mRNA expression in mouse fast-twitch muscles. Experimental Biology Meeting, San Diego, CA. **(poster-NSBRI)**.

Reid, R, T. Towse, **RW Wiseman** and R. Meyer. 2003. Brain activation during muscle recruitment. Int. Soc. Mag. Res. Med. Toronto, Canada. **(poster-NIH,NSBRI)**.

Key Findings from the Kushmerick Team

No report was submitted by Dr. Kushmerick. His project in early 2004

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

ANNUAL TEAM REPORT

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Project 1: Psychosocial Performance Factors in Space Dwelling Groups

PI: Joseph V. Brady, Ph.D. The Johns Hopkins University School of Medicine, Professor
(See address above under Associate Team Lead)

Project 2: Enhancing Team Performance for Exploration Missions

PI: Judith M. Orasanu, Ph.D. NASA Ames Research Center, IHS, Mail Stop 262-4, Bldg. 262,
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Project 3: Self-Guided Depression Treatments on Long-Haul Space Flights

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Project 4: Optical Computer Recognition of Performance Under Stress

PI: David F. Dinges, Ph.D., University of Pennsylvania School of Medicine, Professor
(See address above under Team Lead)

Project 5: Speech Monitoring of Cognitive Deficits and Stress

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Project 6: MiniCog: A Portable and Fast Assessment of Cognitive Functions

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Team Lead

October 22, 2004

Date

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I. ABSTRACT

The NSBRI Neurobehavioral and Psychosocial Factors Team encompasses critical questions from two of the four areas subsumed under Behavior and Human Performance: (1) Human performance failure because of poor psychosocial adaptation, and Human performance failure because of neurobehavioral dysfunction. The Team is charged with conducting research for the development of countermeasures that will ensure that astronaut behavioral health is maintained during prolonged space missions, and that both individual astronaut and crew functioning are effectively optimized. There are 6 ground-based projects making up the current Team. The studies are divided equally between psychosocial and neurobehavioral scientific questions. They cover a considerable breadth of scientific techniques. The overarching focus of all 6 projects is on the impact of stress—individual and interpersonal—on behavioral functions. Collectively, the projects seek to identify (1) the causes of stress, and its consequences for astronaut cognitive, affective and social functioning; (2) techniques to objectively detect stress reactions and performance deficits in individuals and groups in the remoteness of space; and (3) countermeasures to prevent and otherwise mitigate the occurrence of stress reactions and their adverse effects on individual and crew performance. During the past year all projects successfully went through peer review and were selected for continued funding. Project 1 (Brady) and Project 2 (Orasanu) have gathered extensive data on different synthetic task environments, which provide realistic simulations of small-group performance, communication and problem-solving of the kinds required in space flight. These projects are documenting ways to counter the negative impact on team performance from intra-team conflict and stressful conditions. Project 3 (Carter) has completed interviews with astronauts who have long-duration space flight experience and ground control personnel, to develop the content and design of the conflict resolution and depression sections needed for a prototype system. The conflict resolution and depression modules include training content on how to prevent, assess, and manage these problems. Project 4 (Dinges) has completed data acquisition on performance-induced stress and completed prototypical development of a novel optical computer recognition (OCR) algorithm for unobtrusive 3-dimensional tracking of changes in facial expressions during stress. The OCR algorithm development approach is being tested and improved to provide an unobtrusive technique for monitoring stress during performance onboard long-duration spacecraft. Project 5 (Lieberman) has also made significant progress in developing and validating an unobtrusive speech-based computer algorithm for detecting cognitive impairment associated with hypoxia and stress in Everest climbers. Two speech measures have shown promising results. Project 6 (Kosslyn) has completed development of the MiniCog, which is a set of brief cognitive performance tasks on a hand-held device that can be used to quickly assess cognitive capability in remote locations. Validation is underway. In addition to accelerating progress being made on individual projects over the past 12 months, the Team has coalesced collaboratively and now has 12 synergistic collaborations underway across the 6 projects. During the coming year the Team plans to continue the extensive intra-team collaborations to develop convergent validity of approaches and to accelerate the development and transition of pragmatic countermeasures to operational test beds at NASA JSC. The Team is committed to increased interaction with new Behavioral Health and Performance personnel and new Medical Operations personnel at JSC. Team focus on development and delivery of technologies that facilitate psycho-social and neurobehavioral management in space flight, in a manner that is unobtrusive, very astronaut-friendly, minimally demanding of hardware or upmass, and easy to understand, will be maintained. The team will, however, continue to advocate for access to a programmable residential laboratory that permits experimental study of psychosocial issues, as well as access to analog environments that have realistic extreme conditions.

II. INTRODUCTION

The Neurobehavioral and Psychosocial Factors Team was formed in 2000, and funded in 2001. Projects re-competed for continuing funding in 2004. Six were scored high enough by the peer review and management to warrant renewal with continued funding. These projects build on past work and seek to mitigate risks to both individual behavioral health (i.e., neurobehavioral functions) and group processes (i.e., psychosocial functions) during space flight. Specifically, the Team seeks to counter the development of psychosocial risks manifested through inadequate leadership; interpersonal strife or social alienation (e.g., due to gender, culture or status differences); poor group teamwork; lack of crew coordination in problem solving; ineffective communications within the team or with ground controllers; and loss of crew morale. In parallel, other projects on the Team seek to counter the development of neurobehavioral problems manifested through stress reactions; anxiety; depression; loneliness; anger; and neurocognitive impairments.

Unlike some areas of NSBRI research, where there is a single source for the biomedical problem (e.g., microgravity effects on muscle or bone), there are a considerable number of factors in prolonged space flight that could create or contribute to neurobehavioral and psychosocial dysfunctions (e.g., excessively scheduled activities and work requirements, poor physiological adaptation to microgravity; interpersonal strife; perceived risks to health; loneliness for family; inadequate communication with Earth; habitability constraints; radiation). Consequently, the countermeasures being developed through the research by the Neurobehavioral and Psychosocial Factors Team necessarily must cover an array of issues and approaches. The following are the various categories in which countermeasure development is anticipated from the research on the Team.

- 1—Selection criteria for optimal crew cohesion, including culture and gender diversity
- 2—Training for group living; training for flight and ground crew optimal relations
- 3—Guidelines to optimize communication for crew decisions and problem solving
- 4—Technologies for monitoring and early diagnosis of cognitive problems and emotional distress
- 5—Behavioral treatments for stress; affective disorders; and for resolving team conflicts
- 6—Pharmacological treatments for stress; affective disorders and serious neuropsychiatric and neurological reactions
- 7—Habitability strategies for privacy; and work strategies for motivation and performance
- 8—Support for relaxation and leisure activity for enhancing quality of life
- 9—Support for assimilating crews psychosocially and neurobehaviorally after return
- 10—Novel countermeasure opportunities identified by NASA and through new scientific efforts
- 11—Development of a database on the neurobehavioral and psychosocial effects of countermeasures for other biomedical problems in space flight

Ideally, the risks to individual and group behavioral health created by spaceflight are best dealt with through prevention (e.g., a well-integrated crew with optimal pre-flight training and coordination in effective communication, problem-solving, etc.). Therefore one of the focuses of the projects on the Team is to reduce psychosocial risks by identifying the psychological and behavioral features of individuals and small groups that result in optimal behavioral effectiveness under ground-based (analog) conditions comparable to space flight. This approach is taken in the projects directed by Dr. Joe Brady, Dr. Judith Orasanu, and Dr. James Carter, which seek to determine ways to prevent or resolve miscommunication, psychosocial conflict and poor team problem-solving within teams and between space-bound teams and ground controllers.

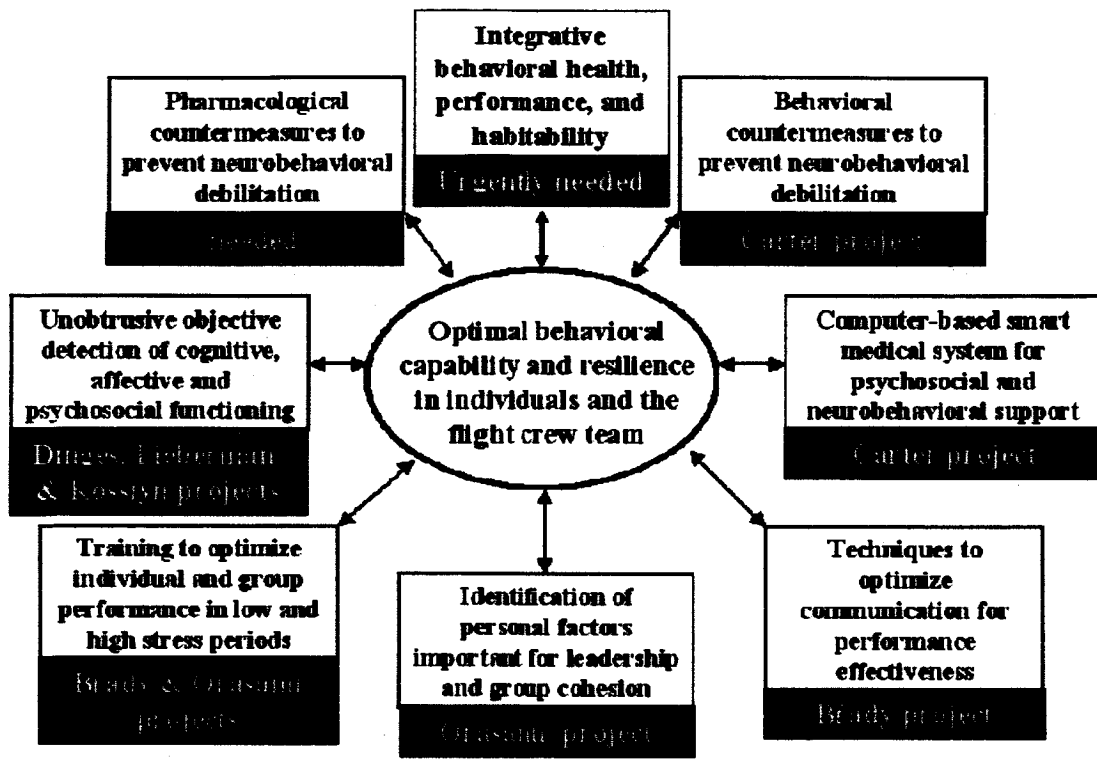
While prophylaxis against the development of neurobehavioral and psychosocial problems is ideal, there is no way to guarantee that preventative strategies alone will suffice. The Team therefore also has a strong focus on early detection and resolution of neurobehavioral problems and psychosocial conflicts. Especially critical is the need for reliable, objective measures of neurocognitive and emotional states and stress reactions. The projects directed by Dr. David Dinges, Dr. Phillip Lieberman, and Dr. Stephen Kosslyn deal directly with obtaining these measures. The emergence of either thought or mood disorders during space flight is likely to be a low-probability event, but if it occurs, it poses very serious risks not only for the individual's behavioral capability but also for the team's performance. Both neurobehavioral and psychosocial problems have occurred in long-duration space flight—with the latter common enough to have resulted in its rating as among the most serious risks (i.e., Type I) in Bioastronautics; consequently, there remains an acute need to establish reliable, objective, unobtrusive methods for confirmation of stable cognitive and emotional functioning during prolonged human space flight. Without such information available to an astronaut, it will be difficult to ensure that appropriate neurobehavioral countermeasures (e.g., behavioral and pharmacological) will be deployed in a manner that maintains behavioral health. There must be redundant ways to ensure that neurocognitive, neurological, and neuropsychiatric problems that develop on orbit (regardless of the cause) are quickly identified and treated before they result in a loss of high-level performance capacity in a crewmember.

The Team also has a major goal of finding optimal ways for crews to use communication modalities and techniques to maintain effective group functioning and problem solving within a flight crew and between the crew and ground controllers, family, and management. Effective communication can help maintain team performance in the face of adversity, and it is one of the best preventative and operational countermeasures for ensuring strong group psychosocial cohesion and performance. Like the development of novel objective, unobtrusive methods and approaches to monitor stress reactions, cognitive state, mood and performance in individual crew members, the establishment of maximally effective communication techniques for all types of contingencies in space flight will have significant relevance to a host of Earth-based problems.

The figure (schematic) below illustrates the Neurobehavioral and Psychosocial Factors Team major research themes and anticipated countermeasure types of the current projects, as well as the projects relevant to each theme. It also shows two areas of continued need for advancing psychosocial and neurobehavioral countermeasures. Together the projects address critical complementary components to the maintenance of behavioral and psychosocial health and capability during long duration missions. The six ground-based projects making up the Neurobehavioral and Psychosocial Factors Team have been underway an average of 42 months.

III. TEAM ACCOMPLISHMENTS

The Neurobehavioral and Psychosocial Factors Team have completed data acquisition on all initial projects and begun new projects. Projects have begun to produce findings at an accelerated rate. Intra-Team collaborations have increased markedly to 12 active collaborations across projects. Production of findings is also increasing. In the past year, the Team was collectively responsible for 14 peer-review publications and dozens of chapters, abstracts and presentations. Progress on each project during the past year is described below.



Project 1. Brady, J. et al. Psychosocial Performance Factors in Space Dwelling Groups.

Key findings and milestones. This project was successfully re-competed for continuation funding during the past year. It addresses critical risks associated with human performance failure due to poor psychosocial adaptation. The goal of the project is to determine the effects of variations in the structure and function of communication channels within and between simulated space-dwelling and Earth-based groups. It uses a distributed interactive multi-person simulation in computer-generated environments, as an experimental test bed for modeling psychosocial performances within and between space-dwelling and Earth-based groups. The project is currently assessing the effects on team communication of changes in (1) communication modalities; (2) positive and punitive reinforcement; (3) time pressure and conflict conditions; (4) training aids and personality; and (5) varying exchanges between crew and ground. The simulation consists of one crew member located (virtually) in a simulated "Orbiter" vehicle that circles the planet overhead, provides remotely-sensed geologic information of the surface, and can provide detailed analyses of any geologic samples collected on the surface; a second crew member is on the planetary surface in a "Rover" vehicle designed to move across the surface, collect and store samples, and transmit sample analysis information to the Orbiter for more detailed analyses; a third crew member is on the planetary surface in a "Lander" vehicle that provides logistic support for the Rover.

Studies over the past year have focused upon the effects of modality constraints and the development of countermeasures to maintain performance effectiveness. Studies in the past year have also shown that functional interchangeability between available communication modes served as an effective countermeasure to communication modality constraints. In the course of studies undertaken over the past year, the effects of simulated failure of the entire conventional communication network (i.e., audio, video, whiteboard mark-up, and text messaging) have been analyzed experimentally in relationship to the innovative utilization of an alternative navigation network as an indexical countermeasure to such severe communication network system failure. Indexical communication depends upon representation of objects and events in terms of their historical relationship to an agent and the context in which concrete actions relevant to that relationship produce effective outcomes. A key finding this past year was that the range of innovative indexical actions (e.g., physical vehicular movements, marking geologic specimens with structural vehicular components, intermittent loading door operation, etc.) it possible to maintain task performance effectiveness comparable to control levels with all components of the communications system network fully functional. The navigation network indexicals were thus found to serve as effective countermeasures under such severely constrained communication conditions. Self-report measures of psychosocial adaptation also provided indications of individual crewmembers affective reactions to the stressful communication constraints and the resulting changes in role functions (e.g. crewmember leadership).

Significant scientific/technology/education papers.

Brady JV, Hienz RD, Hursh SR, Ragusa L, Rouse C, Gasior ED: Distributed interactive communication in simulated space-dwelling groups. *Computers in Human Behavior*, 20, 311-340, 2004.

Hienz RD, Brady JV, Hursh SR, Ragusa L, Rouse C, Gasior ED: Distributed interactive communication and psychosocial performance in simulated space-dwelling groups. *Acta Astronautica* (in press).

Brady JV: Behavioral Health: The Propaedeutic Requirement. *Aviation Space Environmental Medicine* (in press).

New intellectual property; product demonstrations; NASA collaborations. None.

Academic/industry/government partnerships. This project has formal collaborative arrangement with the Simulation Laboratory at Science Applications International Corporation (SAIC) in Orlando, Florida. That facility has been responsible for developing and maintaining all the software for the research simulation test bed. The project investigators also maintain close scientific and intellectual exchange with the NSBRI program and projects at the Johns Hopkins University School of Medicine and the Johns Hopkins Applied Physics Laboratory, which has extensive partnerships with Federal Government Agencies including NASA.

Synergies with other projects. The project has important synergistic relationships with Dr. Orasanu's (Project 2) "Enhancing Team Performance for Exploration Missions", and Dr. Carter's (Project 3) "Self-guided Depression Treatment on Long-duration Space Flights." Together with Dr. Orasanu, Dr. Brady and colleagues are coordinating individual subject evaluation methods (e.g. WinSCAT) as well as outcome measures of performance and psychosocially stressful conditions. Dr. Brady also will be pretesting in the distributed interactive simulation laboratory, Dr. Carter's computer-based Problem Solving Treatment (PST) modules.

Advances toward and testing in flight. Some of the countermeasures beginning to emerge from laboratory studies of communication constraints (e.g. modality interchangeability, indexical innovations) are being proposed for possible use in flight training exercises.

Space and Earth-bound implications of research. Long-duration spaceflight missions will require that small groups of participating individuals operate as integrated teams for extended time intervals under conditions that require effective communication and information exchange for collective problem solving. The development of essential countermeasures to the constraints imposed by the inevitable system failures (physical as well as interpersonal) under such unavoidably stressful conditions must be a critical feature of the research investments made in support of exploration missions beyond Earth orbit. To the extent that the technologies developed to address these problems can generate and evaluate countermeasure efficacy, they can be expected to have an important impact on safety and the quality of life in many Earth-based applied settings.

Project 2. Orasanu, J. et al: Enhancing Team Performance for Exploration Missions.

Key findings and milestones. This project was successfully re-competed for continuation funding during the past year. It addresses critical risks associated with human performance failure due to poor psychosocial adaptation. The project uses a simulated task environment (search and rescue) to study psychosocial processes in team problem-solving. It seeks to determine (1) the effects of task and interpersonal stressors on team interactions and performance and (2) the effects of team composition on team performance; and to identify (3) tools to predict team deterioration and (4) countermeasures models and techniques that support effective team selection, composition, and training.

During the past year, hierarchical linear modeling and path modeling yielded the following key findings. Team performance (task success) suffered in the high task-stress condition but not in

the interpersonal stress condition. Team success depended on team cohesion, which was influenced by the personality of the base camp coordinator (analogous to mission control). If this individual was conventional and tended to avoid the unfamiliar, the team was more cohesive. Individual performance was influenced by crew members' levels of extraversion, neuroticism, and conscientiousness, but only when mediated by team dynamics, specifically by levels of interpersonal support, acceptance, and team collaboration. Preliminary analyses suggest that successful team performance is associated with TEAM-related rather TASK-related aspects of team communications. Specifically, effective team members built on each other's contributions and coordinated their actions rather than directed actions of others.

Significant scientific/technology/education papers.

Kraft NO, Lyons TJ, Binder H: Group dynamics and catecholamines during long-duration confinement in an isolated environment. *Aviation Space Environmental Medicine*, 74, 266-272, 2003.

Kraft NO, Lyons TJ, Binder H: Intercultural crew issues in long-duration spaceflight. *Aviation Space Environmental Medicine*, 74, 575-8, 2003.

Paletz SBF, Peng K, Erez M, Maslach C: Ethnic composition and its differential impact on group processes in diverse teams. *Small Group Research*, 35, 128-157, 2004.

Kraft N, Binder H, Lyons T, Kass R: Interpersonal relationships-types during 264-day confinement in an isolated environment. *Acta Astronautica*. (in press).

New intellectual property; product demonstrations; NASA collaborations. Study is a NASA Lab.

Academic/industry/government partnerships. Investigators from Georgia Institute of Technology and Aptima, Inc. are co-investigators. Licensing agreements have been signed with Harvard University for the use of Dr. Kosslyn's MiniCog (Project 6) and with Wyle for the use of the WinSCAT. In addition, a collaboration was established with Dr. N. Stone (group dynamics expert) of Creighton University, who was a NASA Visiting Faculty Fellow in 2004.

Leveraging of resources. This past year NASA HQ supported hire of a new Ph.D. in the Human Factors Division. Dr. S. Paletz recently completed her Ph.D. (cross-cultural concepts of creativity) and her M.A. (social and personality effects on teamwork) at the University of California-Berkeley. She is devoting approximately half of her time to the project. NASA Airspace Systems and Aviation Safety Program provided additional support for the project's research assistants (Master's level students/graduates) who assist in transcribing and coding facial affect and communication. NASA research on Human and Organizational Risk Models supported by the Engineering for Complex Systems Program (now under the new Exploration Missions Directorate) provided adjunct support for the project to examine risk factors associated with team composition, team communication tools and procedures, and management policies that affect team performance. Funding is anticipated beginning in FY'05 from the Behavioral Health and Performance Program under Dr. Fogelman in the Exploration Missions Directorate at NASA. This effort will complement the ongoing NSBRI research.

Synergies with other projects. This project has collaborations with all five other NBPF projects. As described above in Project 1, Dr. Orasanu is collaborating with Dr. Brady (Project 2) on common measurements of team performance from their synthetic task simulations. The

collaboration with Dr. Carter (Project 3) involves plans to test aspects of the latter's crew conflict management program. With Project 4 (Dr. Dinges) plans are underway to provide videotapes of team members as they engage in stressful tasks, for further development of optical computer recognition of stress. The collaboration with Dr. Lieberman (Project 5) will involve sending audiotapes of speech samples from Project 2 participants under stressful performance demands. The MiniCog from Dr. Kosslyn (Project 6) will be used to assess individual cognitive abilities so that these can be included as covariates in our team performance models of Project 2.

Advances toward and testing in flight. None.

Space and Earth-bound implications of research. This project is determining the inter-relationships between emotion, physiological stress responses, performance, communication, and conflict in teams. Findings from the project will help inform team performance issues in many Earth-based applications (e.g., mission control, engineering support teams). A better understanding of team dynamics and performance is also vital beyond NASA (committees, executive teams, tiger teams, policy bodies, cross-national distributed work teams or co-located groups engaged in important government decision making).

Project 3: Carter, J. et al: Self-Guided Depression Treatments on Long-Haul Space Flights.

Key findings and milestones. This project was successfully re-competed for continuation funding during the past year. It addresses critical risks associated with human performance failure due to poor psychosocial adaptation and human performance failure due to neurobehavioral problems. The goal of the project is to develop a prototypical computer-based "psychosocial support system" for use in pre-flight training and onboard as a countermeasure resource. The primary aim of the final countermeasure system is to minimize psychosocial and neurobehavioral problems through prevention and intervention. The system will provide psychosocial countermeasures through training in the prevention and management of conflicts that can arise in flight and between the astronauts and ground crews. The system will provide neurobehavioral countermeasures through the development of a self-treatment program for mild depression, as well as training in how to recognize depression in one's self and in others. Self-administered standardized measures of psychosocial problems will be included in order to guide users to relevant portions of the system. The system is designed to be used confidentially, with results reported only to the user.

During the past year work was completed on a prototype of the conflict management training portion of a computer-based "smart" medical system for teaching team members how to prevent and reduce psychosocial and neurobehavioral problems in space flight. This prototypical computer-based video system for conflict resolution module has begun to be evaluated by astronauts and mission specialists at NASA JSC. Dr. M. Whitmore, Director of JSC's Usability Testing and Analysis Facility is overseeing this evaluation. Additionally, two ISS crewmembers from recent missions were interviewed for additional content in developing the computer-based modules. Work is now beginning on the smart medical system for self-guided computer-based assessment of (and cognitive treatment of) depression.

Significant scientific/technology/education papers.

Carter JA: Designing a smart medical system for psychosocial support in space. *Aviation Space Environmental Medicine*, (in press).

New intellectual property. The computer-based programs for conflict resolution, as well as for assessment and cognitive treatment for depression are being copyrighted.

Product demonstrations. The prototypical conflict resolution system was demonstrated at the NSBRI retreat, January, 2004. The computer-based program on conflict resolution will also be demonstrated at NASA JSC's "Training for Tomorrow 2004" conference on October 27, 2004.

NASA collaborations. Dr. Carter is currently collaborating with Dr. M. Whitmore at NASA-JSC's Usability Testing and Analysis Facility. Several of the long-duration mission veterans who were interviewed to develop content for the conflict resolution system have also offered to review scripts. Dr. Carter has also closely collaborated with Dr. Holland at NASA JSC. Collaboration will continue with other psychologists and psychiatrists at JSC.

Academic/industry/government partnerships. Collaboration with The Troupe Modern Media Production and Design has been vital to the development of the computer-based prototypes. The Troupe is a multimedia production company that includes staff and facilities for video recording and post-production, audio recording, 2-dimensional graphics, 3-dimensional graphics, and programming. The investigators write and design all elements of the program, and the Troupe executes these designs. Additionally, all media (video and audio) is recorded at the Troupe.

Leveraging of resources. Now based at the Center for Clinical Computing at Beth Israel Deaconess Medical Center, Dr. Carter has access to input from a range of other researchers who are working on related projects dealing with patient-computer interaction. Their comments have led to refinements in the system's design to increase its usability.

Synergies with other projects. Dr. Carter has begun collaborations with Dr. Orasanu (Project 2) and Dr. Brady (Project 1) to test the efficacy of the conflict management module in their simulations.

Advances toward and testing in flight. Project 3 investigators have had discussions with NASA JSC's "laptop computer group," which gave them guidelines to follow in design to ensure that the countermeasure can be implemented. Additionally, they have had informal discussions with NASA operations regarding use of the countermeasure on the ISS. Because the countermeasure requires no additional hardware, is self-contained on a DVD, and is innocuous (poses no risk to its users), it could be implemented on ISS as soon as it is completed and tested to ensure compatibility with other onboard software.

Space and Earth-bound implications of research. Dr. Carter and colleagues are developing a countermeasure that will be deployable on orbit, but it has broad applicability to many Earth-based operations as well. The countermeasure could readily be modified for use by persons working in other isolated environments (military and commercial ships, polar research bases, oil rigs, etc.), as well as for use by the general public in schools, mental health centers, primary care practices, public health centers, social service agencies, HMOs, prisons, and other settings.

Project 4: Dinges, D.F. et al.: Optical Computer Recognition of Performance Under Stress

Key findings and milestones. This project was successfully re-competed for continuation funding during the past year. It addresses critical risks associated with human performance failure due to neurobehavioral problems. The overarching goal of the project is to determine whether optical computer recognition (OCR) algorithms based on changes in facial expressions can discriminate behavioral stress induced by low versus high workload performance demands, and what influence gender, age ethnicity, alexithymia and other personality dimensions have on algorithm discriminability. The computer-based recognition system uses automatic optical tracking of human subjects' subtle anatomical and motor changes in facial expressions during cognitive performance tasks. Video input to the system is provided from experiments on healthy adults exposed to laboratory simulations of varying degrees of workload-based behavioral stressors.

During the past year a major study was completed on 60 healthy adults undergoing behavioral stress induced by low versus high workload performance demands. Bootstrap development of the OCR algorithm was completed using expressive elements of the face identified by a human observer (blind to high- versus low-stressor performance demands). These parameters were used in a Hidden Markov Model to identify high- and low-stressor conditions. The OCR algorithm—developed in the laboratory of the Project Co-P.I., Dr. Dimitris Metaxas (Rutgers University)—relied on changes in mouth and eyebrow regions to accurately discriminate high- from low-stressor performance bouts in 75%-88% of subjects. Additional research begun in the later half of the past year will add facial touching gestures to further improve the OCR detection algorithm, as well as automating application of the deformable masks and OCR algorithms to video footage to increase practical utility.

Significant scientific/technology/education papers.

Goldenstein S, Vogler C, Metaxas D: Statistical integration of DAG deformable models. *IEEE Transactions on Pattern Analysis and Machine Vision* 25(7):801-813, 2003.

Dinges DF, Rider RL, Dorrian J, McGlinchey EL, Rogers NL, Cizman Z, Goldenstein SK, Vogler C, Venkataraman S, Metaxas DN: Optical computer recognition of facial expressions associated with stress induced by performance demands. *Aviation Space Environmental Medicine*, (in press).

New intellectual property. None.

Product demonstrations. A prototypical OCR facial algorithm was demonstrated at the NSBRI retreat, January, 2004.

NASA collaborations. None, but these are anticipated during the next year.

Academic/industry/government partnerships. This project involves collaboration between leading investigators from two separate academic institutions, neither of which could undertake the work alone. Dr. Dinges (University of Pennsylvania)—an expert in behavioral stress and its effects—conducts the behavioral stress induction and data acquisition, while Dr. Dimitris Metaxas (Rutgers University)—an expert in optical computer recognition—analyses the images obtained and develops the OCR algorithm.

Leveraging of resources. None.

Synergies with other projects. Dr. Dinges is also undertaking collaboration with Dr. Lieberman (Project 4) to determine if the voice analysis software can discriminate speech of healthy adults undergoing a high-workload performance stressor versus low-workload (data gathered in Project 4 during the past year); and with Dr. Kosslyn (Project 6) to assess the sensitivity of the MiniCog test battery to fatigue.

Advances toward and testing in flight. The OCR system is not yet ready for flight testing.

Space and Earth-bound implications of research. The results have the potential to identify an objective, unobtrusive, automated method for the recognition, monitoring, and management of the risks of neurobehavioral dysfunction due to work-related stress in spaceflight and in many Earth-based safety-sensitive occupations in which people perform high-workload critical tasks.

Project 5: Lieberman, P. et al.: Speech Monitoring of Cognitive Deficits and Stress.

Key findings and milestones. This project was successfully re-competed for continuation funding during the past year. It addresses critical risks associated with human performance failure due to neurobehavioral problems. The overarching goal of this project is to develop unobtrusive measures of speech acoustics to monitor flight crews for deleterious changes in cognition, personality and emotion arising from damage to the nervous system by cosmic rays during long-term space travel. Such damage is expected to have its earliest effects on several subcortical structures, in particular the basal ganglia, which participate with various cortical regions in "circuits" that regulate cognition, mood, personality and motor control, and the hippocampus, which is critical for a various aspects of memory.

During the past year Dr. Lieberman has studied speech in relation to cognition in climbers on Mount Everest and in patients with Parkinson's disease (PD), a neurodegenerative disorder affecting basal ganglia function. Results obtained support the scientific basis of the proposed system (i.e., the cognitive roles of the basal ganglia and hippocampus), and provide evidence for the practicality of tracking cognitive dysfunction via speech measures. (1) Data on climbers ascending Mount Everest show that cognitive operations that depend on basal ganglia function are susceptible to hypoxia. At higher altitudes climbers exhibit increased sentence comprehension response times, increased errors on the Odd-Man-Out test of cognitive set-shifting, and increased RT on working memory tasks of the MiniCog test battery. Hypoxia also impairs control of speech. At higher altitudes voice-onset time (VOT) separation between voiced and voiceless word-initial stop consonants (e.g., ba vs. pa) decreased and vowel duration increased. (VOT is the time difference between the opening of the supralaryngeal vocal tract and the onset of laryngeal phonation. It depends on the sequencing function of the basal ganglia.) These speech parameters returned toward normal values when climbers descended. This research indicates that speech metrics track cognitive deficits. In addition to VOT, vowel duration correlated significantly with RT on the MiniCog verbal working memory task and showed trends toward significant correlations with spatial working memory and vigilance RTs. Extreme errors on the Odd-Man-Out test and speech deficits were seen in one climber who exhibited a marked personality shift, "perseverating" against all advice in his plan to climb Everest in the face of severe weather. This course of action ultimately led to his death.

In 2004 a sub-group of Everest climbers completed an adaptation of an implicit contextual learning test used to study amnesics with hippocampal damage. Preliminary analyses of Base

Camp data show the contextual learning effect seen in healthy individuals. At higher altitude, however, the climbers showed signs of cognitive amnesia. Their speech is being analyzed.

Significant scientific/technology/education papers.

Lieberman P: *Towards an Evolutionary Biology of Language*. Cambridge, MA: Harvard University Press, in press.

Lieberman P, Morey A, Hochstadt J, Larson M: Mount Everest: A space-analog for speech monitoring of cognitive deficits and stress. *Aviation Space and Environmental Medicine*, in press.

New intellectual property; product demonstrations; NASA collaborations. None.

Academic/industry/government partnerships. Dr. Lieberman has obtained the Palm Corporation's cooperation in supplying us with Palm Pilot PDAs for use in testing climber-subjects on Mount Everest.

Leveraging of resources. For research on sentence comprehension in patients with Parkinson's Disease, Dr. Lieberman has used a head-mounted eye-tracker purchased for general use in the Brown Department of Cognitive and Linguistic Sciences. This technology was acquired from an NSF Integrative Graduate Education and Research Traineeship (IGERT) grant.

Synergies with other projects. Dr. Lieberman has collaborated with Dr. Kosslyn (Project 6) to incorporate the MiniCog Quick Assessment Battery into research on Mount Everest. This field experience resulted in useful feedback that led to modifications of the MiniCog system. Dr. Lieberman is also undertaking collaboration with Dr. Dinges (Project 4) to determine if the voice analysis software can discriminate speech of healthy adults undergoing a high-workload performance stressor versus low-workload (data gathered in Project 4 during the past year). This will provide additional validation of speech analysis algorithms relative to behavioral stressors.

Advances toward and testing in flight. During the past year, Dr. Chris Flynn at NASA JSC has expressed an interest in testing Dr. Lieberman's speech analysis software during space flight.

Space and Earth-bound implications of research. The voice analysis techniques being developed in project 5 for unobtrusively monitoring cognitive status via automated measurement of speech parameters have applications for Earth-based scenarios as well. For example, systems based on these techniques could be used to monitor crews for gradual cognitive effects of hypoxia due to failure of aircraft pressurization systems, which have led to disasters in the past. A speech-based system may be useful in monitoring motor and cognitive dysfunction resulting from stress and sleep deprivation. They can be used to monitor disease state in Parkinson's patients. They may also have value in early detection of other neurological conditions (e.g., Alzheimer's disease). Such early detection would permit clinicians to take maximal advantage of therapies, now under development, that can delay or even arrest further decline.

Project 6: Kosslyn, S.: MiniCog: A Portable and Fast Assessment of Cognitive Functions

Key findings and milestones. This project was successfully re-competed for continuation funding during the past year. It addresses critical risks associated with human performance failure due to neurobehavioral problems. The goal is to develop a set of brief performance tests on a handheld

device that will be computerized versions of standard tasks that tap a range of basic cognitive abilities. The performance tests being developed were selected from among standard tasks that tap abilities necessary for performing complex cognitive operations of the kind astronauts encounter in spaceflight.

The project has had two types of milestones—those pertaining to developing the MiniCog program and those pertaining to use of the cognitive tests (MiniCog Quick Assessment Battery) delivered by the program. During the past year, substantial progress has been made in both areas. With regard to the technology, (1) the MiniCog was updated by improving the user interface to allow more information to be collected prior to the start of a testing session and to make navigation through the program simpler; (2) the software was upgraded to run on newer Palms, and the stimuli were re-created at a higher resolution to match the capabilities of the new hardware; (3) the data backup method was made more secure; and (4) greater flexibility was added to specifying the timing of stimulus presentation. Regarding the cognitive testing, (1) data collection was completed on a study using performance on the test battery as a predictor of performance on more complex and longer tasks; and (2) data collection was begun on a study of the effects of fatigue on performance, and the potentially ameliorating effects of caffeine.

Significant scientific/technology/education papers.

Shephard JM, Kosslyn SM: MiniCog: A "Blood Pressure Cuff" for the mind. *Aviation Space Environmental Medicine*, (in press).

New intellectual property. A utility patent was filed in August, 2004, for "A system and method for on-site cognitive efficacy assessment" (provisional patent serial no. 60/494883).

Product demonstrations. The prototypical MiniCog Quick Assessment Battery was demonstrated at the NSBRI retreat, January, 2004.

NASA collaborations. None, but these are anticipated during the next year.

Academic/industry/government partnerships. Dr. Kosslyn has licensed MiniCog Quick Assessment Battery to other researchers in neuropsychology and medicine to learn whether the test battery can be useful in diagnosing brain damage and head injuries. He is also planning a collaboration with Drs. L.A. Vaughn and N. Rader at Ithaca College to study MiniCog Quick Assessment Battery in researchers over-wintering at MacMurdo Station in Antarctica.

Leveraging of resources. None.

Synergies with other projects. Dr. Kosslyn is collaborating with three other NBPF projects. Dr. Lieberman (Project 5) administered the MiniCog Quick Assessment Battery to climbers on Mt. Everest during the past year as part of studies of the effects of high altitude on cognitive abilities. Dr. Orasanu (Project 2) has begun administering the MiniCog Quick Assessment Battery as an individual differences measure in her study of distributed team decision making. Dr. Dinges (Project 4) is beginning administration of the MiniCog Quick Assessment Battery in studies of chronic sleep restriction similar to that experienced by astronauts.

Advances toward and testing in flight. Plans are being developed to work with flight surgeons and staff at Wyle Laboratories for flight studies in the near future.

Space and Earth-bound implications of research. In addition to providing valuable feedback to astronauts in space regarding their current cognitive performance capabilities, the MiniCog Quick Assessment Battery has a range of possible uses on Earth. It has advantages over standard task batteries and typical psychological testing in that the tests are brief and the method of administration is compact, portable and inexpensive. These characteristics make it practical in a wide range of settings where there are questions regarding neurocognitive capability.

IV. TEAM PLANS

The Neurobehavioral and Psychosocial Factors Team plans to advance the following six objectives in the coming year.

- (1) Continue the extensive intra-team collaborations among projects to develop convergent validity of approaches and to accelerate the development and transition of pragmatic countermeasures to operational test beds at NASA JSC.
- (2) Continue and increase interaction between NBPF Team projects and new Behavioral Health and Performance personnel and new Medical Operations personnel at NASA JSC (i.e., replacements for Dr. Chris Flynn, Dr. Al Holland), as well as others at JSC and Wyle.
- (3) Maintain the Team focus on development and delivery of technologies that facilitate psychosocial and neurobehavioral management in space flight, in a manner that is unobtrusive, very astronaut-friendly, minimally demanding of hardware or upmass, and easy to understand.
- (4) Continue to find ways to accelerate psychosocial and neurobehavioral countermeasure research by getting access to a programmable residential laboratory for projects that experimentally address interpersonal interactions, culture and gender, and innovative techniques for evaluating group processes. Through such a simulation chamber it would be possible to experimentally assess habitability, behavioral health, and performance effectiveness for long-term habitation in space environments. Biomedical monitoring in simulated stress conditions can also be assessed.
- (5) Continue to advocate the addition to the Team of input from at least one observational laboratory in an analog extreme environment, likely the Arctic or Antarctic. At present no project meets this need. The inclusion of a realistic extreme environment would enable the assessment of factors relevant to long-duration missions in actual conditions of danger and thermal stress. This is particularly important in considering that two-person teams would likely leave the space habitat to carry out periodic explorations on the Mars surface.
- (6) Encourage projects focused on the assessment of psychopharmacological agents in microgravity conditions, behavioral measures and genetic markers that could be used for "select-in" screening of astronauts for long-duration missions, and integrative research on behavioral health, performance, and habitability.

The following are the plans for the specific NBPF Team projects.

Project 1. Brady, J. et al. Psychosocial Performance Factors in Space Dwelling Groups.

During the next 12 months, the Project focus will be extended to evaluate the effects of communication time delays on crew performance effectiveness and psychosocial adaptation. The

parameters of these effects will be defined operationally with the objective of developing effective countermeasures to poor psychosocial adjustment and the risk of human performance failure. Research will also be directed toward an evaluation of incentive systems and potentially stressful competitive interactions within and between simulated crew members and Earth-based control centers as these conditions contribute to impairment of individual and group performance effectiveness. It is also anticipated that over the next year, progress will be made on the required modification of the Java-based software extending the distributed interactive simulation test bed to enable internet participation of geographically remote and culturally diverse research groups.

Project 2. Orasanu, J. et al: Enhancing Team Performance for Exploration Missions.

A second baseline study will be conducted next year using the recently reconfigured multiplayer simulation task. Instead of an inherently cooperative task (search-and-rescue), the new task (search for lost scientific archeological artifacts in Antarctica) has both competitive and cooperative goal structures. Data collection will include gender- and culturally-diverse teams. Several new measures will be added including assessment of cognitive functions using the MiniCog and the WinSCAT; assessment of cultural values, cultural cognitive styles, and cultural intelligence, using new scales for these factors; assessment of collective and individual efficacy and group climate using psychological scales; and individual difference measures of personality, conflict styles, and, leadership using standardized psychological assessments.

Project 3: Carter, J. et al: Self-Guided Depression Treatments on Long-Haul Space Flights.

In the next year the conflict resolution module will be finalized and the depression assessment and intervention module will be developed. When multimedia production of the depression content is complete, the depression self-treatment portion will be evaluated in a randomized controlled trial.

Project 4: Dinges, D.F. et al.: Optical Computer Recognition of Performance Under Stress

During the next year a second experiment using performance demands to induce stress will be begun to determine whether OCR based on changes in facial expressions and facial touching can reliably identify low- versus high-stressor conditions; and what influence facial edema, gender, age, ethnicity and alexithymia have on algorithm discriminability. The accuracy of the OCR algorithm to detect stress will be evaluated both with and without facial edema, which occurs in microgravity. The optically-based analysis of facial characteristics will consist of three different levels of representation that depend on the level of motion detail extracted. The first algorithm will consist of face detection, gross movement analysis and facial landmarks; the second will consist of constructing and representing a deformable face model; and the third will involve the computational detection of whether a subject is experiencing stress in response to workload.

Project 5: Lieberman, P. et al.: Speech Monitoring of Cognitive Deficits and Stress.

During the coming year, speech analysis methods will be studied relative to performance task-induced stress (in collaboration with the Dinges' Project and the Kosslyn Project). Correlations between speech measures and physiological and facial analysis measures of stress will also be explored. In spring 2005, data acquisition in Everest climbers will begin again to further enlarge the existing database, and to permit replication and refinement of voice analyses. Work on computational techniques for automatically deriving speech metrics will continue.

Project 6: Kosslyn, S.: MiniCog: A Portable and Fast Assessment of Cognitive Functions

The MiniCog Quick Assessment Battery will be evaluated in a number of studies ongoing in other laboratories during the coming year including fatigue studies (Dinges Lab) and analog

environments (Lieberman Everest studies), as well as in simulated task environments (Orasanu Lab). In addition, the MiniCog will be compared to other test batteries including the WinSCAT; Complex Cognitive Assessment Battery; and the Unified Tri-Service Cognitive Performance Assessment Battery. It will also be evaluated for its prediction of a “real world” simulation task (i.e., laser surgery) under fatigue and caffeine conditions. Other studies will assess the effects of performance anxiety (e.g., public-speaking stress) on MiniCog performance. A feature will be added to the MiniCog that allows a user to predict performance on each task. Throughput will be added to the scoring algorithm, and the capability for presenting auditory stimuli will be added.

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

Research Team Annual Report October 22, 2004

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Projects and Principal Investigators

Projects initiated in 2003:

Visual Orientation, Navigation and Spatial Memory Countermeasures (3/31/2004 -4/29/2007)
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Development of a Gait Adaptability Training Program as a Countermeasure for Postflight Locomotor Dysfunction (10/01/04 – 11/29/2008) Jacob J. Bloomberg, Ph.D, NASA Johnson Space Center, (281) 483-0436 jacob.j.bloomberg1@jsc.nasa.gov

Pharmacotherapeutics of Intranasal Scopolamine, Lakshmi Putcha, PhD, NASA Johnson Space Center, (281) 483-7760, lputcha@ems.jsc.nasa.gov

Sensorimotor Adaptation Following Exposure to Ambiguous Inertial Motion Cues (9/01/2004-10/30/2008), Scott J. Wood, PhD., Universities Space Research Association, (281) 483-7294, scott.j.wood1@jsc.nasa.gov,

Neurovestibular Aspects of Short-Radius Artificial Gravity: Toward a Comprehensive Countermeasure (4/01/04-5/30/2007). Laurence R. Young, Sc.D., Massachusetts Institute of Technology (617) 253-7759, lry@space.mit.edu

Completed 2000-2003 projects:

Visual Orientation and Spatial Memory: Mechanisms and Countermeasures (10/1/2000-7/31/2004)
Charles M. Oman, Ph.D., Massachusetts Institute of Technology , (617) 253-7508, coman@mit.edu

Neuro-Vestibular Aspects of Artificial Gravity Created by Short-Radius Centrifugation. (10/01/2000-2/29/2004) Laurence R. Young, Sc.D., Massachusetts Institute of Technology (617) 253-7759, lry@space.mit.edu

Concluding 2000-2004 projects (final reports not yet due):

Context-Specificity and Other Approaches to Neurovestibular Adaptation (10/1/2000 –9/30/2004)
Mark J. Shelhamer, Sc.D., Johns Hopkins University School of Medicine (410) 614-6302
mjs@dizzy.med.jhu.edu.

Understanding Full-Body Gaze Control During Locomotion (5/01/2001 – 8/31/2004), Jacob J. Bloomberg, Ph.D, NASA Johnson Space Center, (281) 483-0436, jacob.j.bloomberg1@jsc.nasa.gov

Advanced Techniques to Assess and Counter Gait Ataxia (10/01/2000 – 8/31/2004)
Conrad Wall III, Ph.D., Massachusetts Eye and Ear Infirmary , (617) 573-4160, cwall@mit.edu

Modification of Eccentric Gaze-Holding (8/01/2001 - 10/31/2004), Millard F. Reschke, Ph.D, NASA Johnson Space Center (281) 483-7210 millard.f.reschke@jsc.nasa.gov

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One signed original, singled-sided copy with no binding, suitable for reproduction plus one electronic copy on CD in Microsoft WORD for PC .

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I. ABSTRACT

Returning astronauts almost universally report that disorientation and perceptual illusions and space motion sickness in-flight, and re-entry and postlanding disorientation, nausea, and locomotion problems are the most overt physiological problems of space flight. NSBRI's Neurovestibular Adaptation research program is aimed at developing scientifically-based countermeasures against these problems, which are generally most acute after G transitions. Unfortunately the latter always come at times when physical and cognitive performance is critical for safety and mission success. Postflight symptoms have generally been more severe after 3-5 month Mir and ISS flights than on 1-2 week Shuttle missions, demonstrating that some components of neurovestibular adaptation to 0-G take place over time scales of months, rather than weeks. EVA and tele-operation of robot arms and vehicles have become increasingly common during the construction of the ISS, and these also represent significant sensory-motor integration challenges. This year, NASA reorganized to formally plan for very long duration exploration missions to the Moon and Mars. Sensory-motor problems are anticipated whenever astronauts must make a sudden transition from 0-G to the partial G planetary environments, or from 0-G to an artificial gravity environment.

During the past year, the NSBRI Neurovestibular Adaptation Team research portfolio has consisted of 6 projects originally selected in 2000, and 5 projects initiated in the spring of 2004. All address risks identified by the NASA Bioastronautics Roadmap, though the projects selected this year are more specifically countermeasures development oriented. The current team consists of 47 investigators from 22 institutions. Since its formation in 1997, more than 150 journal articles, book chapters, abstracts and manuscripts have appeared, including more than 50 peer reviewed journal articles. Results of most of the year 2000 team projects appeared this year in two special issues of the Journal of Vestibular Research. The first specifically highlighted NSBRI neurovestibular research results, and the second was devoted to scientific papers presented at the Sixth Symposium on the Role of the Vestibular Organs in Space Exploration. This meeting was organized and co-sponsored by our NSBRI team.

Our team is currently focusing on development of countermeasures, and significant research progress has been made in seven areas. These include: Short radius, intermittent artificial gravity; Gait adaptability training; Locomotion assessment techniques; Dynamic visual acuity tests; Intranasal anti-motion sickness drugs; Preflight orientation training methods; and Inflight spatial disorientation reduction via improved crew operational, workstation layout, and architectural design techniques. Several of these are currently being implemented for use in pre-/posflight training and/or flight investigations. This report concludes by detailing eleven team objectives for the coming year.

II. INTRODUCTION

In September, 2004, consideration was given to renaming NSBRI's "Neurovestibular Adaptation" research team as the "Sensory-Motor Adaptation" research team, reflecting the importance of finding countermeasures not only to those risks explicitly related to vestibular function, but also more generally for those related to multi-sensory perception and control of orientation and movement, neural coordination of movement including posture and locomotion, and autonomic and emetic function. A final decision has not been made. This section summarizes of the team's organization and activities, showing relationships among the projects and with other teams, the risk areas and enabling questions on the Bioastronautics Critical Path Roadmap addressed by the team, and thus the ways in which the team supports the Vision for Space Exploration.

During 2004, the NSBRI Neurovestibular Adaptation research team consisted of 47 investigators from 22 institutions, with 26 from 8 institutions continuing into 2005.

The research portfolio consists of 11 research projects listed in Tables 1 and 2 below. Of these, five projects are new this year (Table 1), and were selected in January through NRA 03-OBPR-04.

Table 1: Principal Countermeasures and [CRL] by Project and Risk	Bioastronautics Critical Path Neurovestibular Risks:		
	Vertigo, Spatial Disorientation and Perceptual Illusions	Impaired Movement Coordination Following G- Transitions	Motion Sickness

[illegible]

¹ A 7th project from the June 2000 selection, Dr. Dornhoffer's 2 year investigation of anti-motion sickness

Bioastronautics Critical Path Roadmap enabling questions (Eqs) for the discipline addressed by the projects are shown in Table 3:

Table 3: Bioastronautics Critical Path Roadmap Neurovestibular Enabling Questions (Priorities by mission type, 1 high)	Oman - Orientation Countermeasures	Bloomberg - Gait Adaptability	Pulcha - Intranasal Scopamine	Wood - Sensorimotor Adaptation Countermeasures	Shelhamer - Context Specific Adaptation	Bloomberg - Full Body Gaze Control	Well - Gait Alaxia	Reschke - Eccentric Gaze Holding	Oman - Visual Orientation	Young - Short Radius AG
What are the physiological bases for spatial disorientation, perceptual illusions, and vertigo? [ISS 1 Moon 1, Mars 1]										
What individual physiological and behavioral characteristics contribute to the large inter-individual differences in neurovestibular symptoms and signs? [ISS 1 Moon 1, Mars 1]										
What is the physiological basis for context-specific-adaptation? [ISS 1 Moon 1, Mars 1]										
What are the physiological bases for disruption of balance, locomotion, and eye-head coordination following g-transitions? [ISS 1 Moon 1, Mars 1]										
What are the physiological mechanisms that trigger vomiting in space motion sickness? [ISS 1 Moon 1, Mars 1]										
What combinations of visual, vestibular, and haptic cues cause spatial disorientation, perceptual illusions, and vertigo during and after g-transitions? [ISS 2 Moon 2, Mars 2]										
To what extent can neurovestibular adaptation to weightlessness and/or AG take place in context-specific fashion, so crewmembers can be adapted to multiple environments and switch between them without suffering disorientation or motion sickness? [ISS 2 Mo]										
What pre-flight training techniques (e.g. virtual reality simulations, parabolic flight) can be used to alleviate the risks of spatial disorientation, perceptual illusions, and vertigo as astronauts launch, enter, and adapt to 0-G? [ISS 2 Moon 2, Mars 2]										
What objective assessment techniques can be used to determine crew readiness to return to normal activities following g transitions? [ISS 2 Moon 2, Mars 2]										
What is the physiological basis of the emetic linkage between vestibular and emetic centers? [ISS 2 Moon 2, Mars 2]										
Can g-transition-related spatial disorientation, perceptual illusions, and vertigo be predicted from mathematical models? [ISS 3 Moon 3, Mars 3]										
What individual physiological and behavioral characteristics will best predict susceptibility and adaptability? [ISS 3 Moon 3, Mars 3]										
What in-flight training techniques (e.g. virtual reality simulations, treadmill with vibration isolation system, artificial gravity) can be used to alleviate the risks of vertigo, disorientation, and perceptual illusions as astronauts land and (re)adapt t										
How can voluntary head movements during entry and landing be used to accelerate re-adaptation? [ISS 3 Moon 3, Mars 3]										
Is adaptation to the lunar gravity environment sufficient to reduce incidence of landing vertigo upon return to Earth? [ISS N/A, Moon 3, Mars N/A]										
How can traditional clinical vestibular rehabilitation techniques be employed to usefully accelerate re-adaptation following g-transitions? [ISS 3 Moon 3, Mars 3]										
Can disruption of balance, locomotion, and eye-head coordination following g-transitions be predicted from mathematical models? [ISS 3 Moon 3, Mars 3]										
What level of supervisory control will mitigate the landing vertigo risk in landing on the Moon, Mars, and Earth? [ISS 4 Moon 4, Mars 4]										
What in-flight training techniques (e.g. virtual reality simulations, treadmill with vibration isolation system, artificial gravity) can be used to alleviate the risks of space motion sickness as astronauts land and (re)adapt to Earth, Moon, or Mars gravi										
Is adaptation to the lunar gravity environment sufficient to reduce incidence of motion sickness upon return to Earth? [ISS N/A, Moon 4, Mars N/A]										
Is adaptation to the lunar gravity environment sufficient to reduce incidence of sensory-motor balance and coordination problems upon return to Earth? [ISS N/A, Moon TBD, Mars N/A]										
What AG exposure regimens (G-level, angular velocity, duration, and repetition) will ameliorate the bone, muscle, cardiovascular, and vestibular deconditioning associated with hypogravity during surface operation phases of a mission? [ISS N/A, Moon TBD, M]										
What AG exposure regimens (G-level, angular velocity, duration, and repetition) will ameliorate the bone, muscle, cardiovascular, and vestibular deconditioning associated with hypogravity during transit phases of a mission? [ISS N/A, Moon N/A, Mars TBD]										
What objective assessment techniques can be used to determine crew readiness to return to normal activities following g transitions? [ISS TBD, Moon TBD, Mars TBD]										
How can pre-flight or in-flight sensory-motor training or sensory aids improve post-landing postural and locomotor control and orthostatic tolerance? [ISS TBD, Moon TBD, Mars TBD]										
To what extent can crew "learn how to learn" by adapting to surrogate sensory-motor rearrangements? [ISS TBD, Moon TBD, Mars TBD]										
What are the relative contributions of sensory-motor adaptation, neuromuscular deconditioning, and orthostatic intolerance to post-flight neuro-motor coordination, ataxia, and locomotion difficulties? [ISS TBD, Moon TBD, Mars TBD]										

drug effectiveness, ended more than a year ago, and is therefore not included in this 2004 report.

III. TEAM ACCOMPLISHMENTS

In the context of countermeasure development, progress of programmatic significance during the year ending September 30, 2004 relative to each of the three major sensory-motor adaptation risks is summarized below:

1. Risk of vertigo, spatial disorientation, and perceptual illusions.

Progress has been made by 5 projects:

- Dr. Oman's projects have explored the origins of spatial disorientation, direction and height vertigo, and related navigation problems. The group has employed a fully furnished "tumbling room" and virtual reality techniques. They demonstrated previously effects of scene symmetry and the relative orientation of familiar objects on perceived self-orientation are much larger than previously thought, based on classic experiments by Mach, Witkin and others. For example, they showed that 75% of gravitationally supine subjects experience the illusion of being gravitationally upright or inverted when presented with a compelling visual scene with visual cues to the vertical aligned with their body axis. Such stimuli are believed to trigger Visual Reorientation Illusions (VRIs) in astronauts. This year the group has also developed two important new methods to quantify the direction of the perceived vertical (exploiting the "shape-from-shading" illusion) and to assess the strength of head movement contingent oscillopsia (by adjusting the relative movement of a surrounding virtual scene). In related experiments, they studied the preferred firing direction of limbic head direction cells in rats, which is believed to code the animal's sense of direction. They demonstrated that directional tuning continues in 0-g, but occasionally shows a distinct "disorientation" response state, which may correspond to Type II/III spatial disorientation in humans. Sudden reversals of preferred direction were also noted, which may correspond to VRIs. Although much is already known about human terrestrial (2D) navigation in buildings, little is known about 3D abilities, when body orientation is unconstrained by gravity. The group has pioneered the use virtual reality techniques to study how humans develop 3D cognitive mental models ('spatial frameworks') and shown a correlation in individual performance with mental rotation abilities. The research shows why preflight training in 1-g modules not physically oriented in their actual flight configuration caused direction vertigo and navigation problems in Shuttle, Mir and ISS crews in orbit. The research provides a clear rationale for providing crews with interactive visual experience with space vehicle interiors using virtual reality techniques prior to actual spaceflight as a countermeasure for disorientation, and provides methods for assessment of 3D spatial abilities. This year the group is doing proof-of-concept testing on such a training paradigm (CRL5).

- Dr. Young's projects focus on the neurovestibular effects of short radius, intermittent Artificial Gravity (AG) produced by centrifugal force in a rotating device. Unfortunately, as generations of amusement park riders have discovered, out-of-rotation-plane head movements create an unnatural stimulus to the semicircular canals of the inner ear, causing strong vertigo, spatial disorientation, perceptual illusions and motion sickness. Also, when limbs are moved, physical Coriolis forces deflect them in unexpected ways. AG has long been seen as a potential multi-system countermeasure for Exploration class missions, since it potentially concurrently effective against bone, muscle, and cardiovascular deconditioning. In principle AG could also be used to preadapt the otolith organs to postlanding (e.g. Moon, Mars or Earth) G levels as a neurovestibular countermeasure. Interest in AG has increased this year as a result of NASA's new vision for exploration class missions. From the point of view of mission architecture and vehicle design, the key questions are: 1) is some of AG necessary, or are single-system countermeasures adequate. 2) If AG is required, how much G is sufficient, and can it be delivered intermittently, for example on a relatively short radius centrifuge inside the vehicle, or is continuous rotation of all or part of the vehicle required? 3) Can subjects learn to tolerate the neurovestibular side-effects caused by short radius devices? Engineers naturally prefer the short radius intermittent AG approach, since the components are smaller. The choice between the short-radius/intermittent vs. the large-radius/continuous approach needs to be made by the end of the decade, in order to establish the design requirements for the Crew Exploration Vehicle used to transit to both the Moon and Mars.

Dr. Young's AG projects have extended the pioneering AG studies by Dr. Graybiel's group at Pensacola in the 1960s, by focusing on short radius intermittent AG, and by including objective measures of concurrent adaptation of the vestibulo-ocular reflex as well as adaptation of reaching arm movements. Experiments have been conducted in a short-radius centrifuge at MIT, a medium radius centrifuge at Brandeis, and a Rotating Chair at Mt. Sinai. Dr. Young and his team have shown that approximately 75% of normal subjects can tolerate single axis head movements at 23 RPM on a short radius centrifuge, and that incrementing the RPM while adapting reduces symptoms. They demonstrated that motor adaptations to reaching arm movements are rapid, that VOR adaptation requires a visual scene, whereas subjective sensations adapt in the dark. During adaptation the angular VOR time constant shortens, and that adaptation transfers to some degree across two different directions of centrifuge rotation. A correlation between VOR time constant and motion sickness susceptibility has been observed. Recent data also suggests that adaptation to right quadrant head movements generalizes to the other

direction within a few minutes. These findings are important since they suggest that most astronauts could successfully adapt to high angular velocity, short radius centrifugation, where the crewmember typically sits in a seat, and remains facing in a specific direction. Thus there appears to be no insurmountable neurovestibular problem preventing the application of short radius, intermittent AG against bone, muscle, or cardiovascular deconditioning.

- Dr. Reschke's fundamental research project on gaze stability demonstrated that during eccentric gaze holding the time constant of centripetal drift is dependent on head gravitational position in normals and patients. Further tests are planned to examine gaze stability after centrifugation. The hypothesis is that gaze instabilities and square wave jerks which have been reported by Russian colleagues may be due to G-dependent functional changes in the final oculomotor integrator. (CRL 3).
- Dr. Shelhamer's basic research project – which concludes this fall – demonstrated the ability of the saccadic system to adapt in a context specific manner dependent upon G level, head orientation, vertical eye position and – most recently – on time between training presentations. More recently he has focused on measurement of the gain of the oculomotor response to transient head motions (lateral heaves and fore-aft surges), which might form the basis of a tool for postflight assessment of otolith and cerebellar function.

2. Risk of impaired movement coordination following G-transitions.

- Dr. Bloomberg's project completed development of sensitive tests of near and far dynamic visual acuity during treadmill locomotion, in subjects with both normal vision and while wearing x 0.5 minifying lenses, and at distances of both 0.5 and 4 meters. They found that subjects are able to modify full-body segmental kinematics in order to reduce head perturbations. The acuity test has been integrated into an "integrated treadmill locomotion test" that is currently being used for pre- and post-flight testing of astronauts (CRL 9). New integrated data collection and analysis procedures were developed this year. Dr. Bloomberg's new NSBRI gait adaptability research project – getting underway this fall - will provide important 1-G proof-of-concept data demonstrating the theory of "adaptive generalization" as applied to improvement of postflight movement coordination : Do systematic variations in locomotor challenge during treadmill exercise (e.g. variations in visual scene motion, body loading, or walking speed) improve overall postflight locomotor function, as quantified using the integrated treadmill locomotion test, and also an ad-hoc functional mobility test ? The adaptive generalization concept is concurrently being evaluated as part of a pre/postflight ISS experiment ("Mobility"; see below).
- Dr. Wall's project developed a locomotion perturbation device (balance disturber, or "Balder"), and quantified how responses differed between unilateral vestibular hypofunction patients and normals. A portable version

of the device was built last year and is now being evaluated in Dr. Bloomberg's laboratory. (CRL 7) Dr. Wall's laboratory has also developed a wearable prototype multi-axis vibrotactile balance aid. With NSBRI support Dr. Wall investigated the use of a vibrotactile balance aid to recover from anterior-posterior balance disturbances while standing. A proposal for continuation of the project is currently under review.

- These locomotion and visual acuity assessment techniques will be among those under evaluation for use in postflight Clinical Status Evaluation (CSE). (CRL7).

3. Risk of motion sickness:

- Dr. Putcha has been developing intranasal formulations of promethazine and scopolamine for several years. This year she initiated a new NSBRI sensory motor adaptation team project to determine the pharmacodynamics, cognitive side effects, and dose-response effectiveness of intranasal scopolamine to off-vertical axis rotation. Intranasal administration has advantages for "rescue therapy" as compared to orally administered drugs because they are directly absorbed through the nasal mucosa. Her intranasal promethazine formulation has been in development through a NSBRI Smart Medicine Team project. Scopolamine is thought to be less sedating, but has other side effects which must be assessed. Dr. Shelhamer's concluded his series of parabolic flight experiments investigating possible changes in ocular vertical alignment (skew) which might cause diplopia and contribute to space sickness. (CRL 2). A final report is due this fall. Dr. Putcha's pharmacology expertise provides an important new resource for our team.
- Dr. Reschke prototyped a set of electronic LCD shutter glasses which reduce retinal slip and the stimulus for motion sickness when concurrently wearing prism goggles. He recently obtained separate NASA funding to evaluate the effectiveness of the shutter glasses against motion-induced nausea (CRL 4).

Synergies, NASA Collaborations, advances toward testing in flight:

Dr. Bloomberg's project has major scientific and technical overlap with the ISS HRF experiment "Mobility". In the pre-/postflight version of the experiment, crewmembers in the experimental group undertake a pre-flight practice regimen involving systematic variation in locomotor challenges during treadmill exercise, including visual scene, body loading, and treadmill speed changes. Moving visual stimuli are provided by a flat panel display mounted on the treadmill. Subject performance in an integrated treadmill test and a functional mobility tests is compared with a control group. Hardware to adapt the ISS treadmill to support an in-flight adaptive generalization training countermeasure is also in development, which could fly in 2005.

Dr. Young's project team is also participating in a preliminary NASA-JSC interdisciplinary 1-g bed rest study to determine whether intermittent (1 hour of 1G centrifugation per day, combined with exercise) short radius centrifugation is effective in reducing bone loss. The study will be conducted at UTMB. If results are encouraging, the second phase of this project will involve international participation, and assessment of countermeasure effectiveness against bone, muscle, and cardiovascular effects, and more complete investigation of neurovestibular issues.

Dr. Oman's project has significant scientific and technical overlap with the ISS HRF experiment "VOILA" (Visuomotor and Orientation Investigations in Long Duration Astronauts), an international experiment using virtual reality techniques and haptic stimulus devices to study human orientation and eye-hand motor coordination on long duration ISS flights. The experiment is an outgrowth of an earlier Shuttle/NeuroLab study. Goals include assessment of susceptibility to VRIs in flight, and the effectiveness of a haptic countermeasure (0.4 G axial body loading produced by constant force springs) on visually induced disorientation. VOILA is currently in development by MIT and the College de France, and is supported by NASA and CNES. Dr. Oman and Dr. Alain Berthoz are Principal Investigators, and could fly as early as 2007.

Dr. Oman assisted Dr. Marshburn in developing a series of short inflight medical debriefing questions for ISS Expedition 7 Science Officer Ed Lu. Lu's journal (http://spaceflight.nasa.gov/station/crew/exp7/luleters/lu_letter12.html) includes astute observations on spatial disorientation problems.

Drs. Oman, Solomon, Clark, and Wood obtained NSBRI Director's seed funding for a student project to study "Wobblies" vertigo among elite aerobatic pilots. Interest in Wobblies derives from the presumed common etiologic mechanism with Benign Paroxysmal Vestibular Vertigo caused by displaced otoconia among vestibular patients, and possibility certain acute cases of landing and postlanding vertigo after spaceflight. At the invitation of the International Aerobatic Club, Drs. Oman and Solomon attended the US National Aerobatic Championships in Denison, TX, and gave three briefings on the etiology of Wobblies vertigo, and showed competitors how some cases can be diagnosed and treated canalith repositioning maneuvers. Since that time, at least one competitor has tried the procedure, obtained relief, and returned to do well at the highest levels of competition. An article on Wobblies for Sport Aviation is in preparation, co-authored with several aerobatic pilots.

Industrial Spin-offs:

The Virtual Reality Utilities (VRUT) software developed with partial NSBRI support by Dr. Andrew Beall at MIT for Dr. Oman's projects is now in use at more than two dozen sites, and has recently been commercialized and further development by WorldViz, Inc., and is being licensed as "Vizard" (<http://www.worldviz.com>). The enhanced version is now being used in both Dr. Bloomberg and Dr. Oman's NSBRI projects, and also related ISS flight experiments.

Dr Putcha is discussing FDA regulatory requirements for development of her scopolamine preparation and exploring CRO agreements with pharmaceutical research firms to gear up for phase II studies.

Dr. Reschke's project is working with the NASA-JSC Office of Technology Transfer and Commercialization (Kelly Currin) to explore commercial interest in his LCD shutter glass device for prevention of motion sickness.

Other significant milestones:

- a) Appointment of Dr. Jacob J. Bloomberg of NASA JSC as Associate Team Leader.
- b) Revision of Neurovestibular risks on Bioastronautics Critical Path Roadmap, including citations to relevant literature.
- c) Publication of special issue of Journal of Vestibular Research devoted to NSBRI neurovestibular research in late fall 2003, and a second JVR special issue in the spring of 2004, containing papers presented by NSBRI and other neurovestibular researchers at the 6th Symposium on the Role of the Vestibular Organs in Space Exploration in 2002.
- d) "Next Steps in Space Neurovestibular Research" symposium session at the Barany Society Meeting in Paris, July 9, 2004, organized by Drs. Oman, Paloski and Black. The Bloomberg, Oman, Reschke, Shelhamer, Wall, and Young projects presented at the Barany meeting.

IV. TEAM PLANS

Eleven objectives for the coming year are:

1. Continue development of countermeasures developed by team. This is the Team's first priority. We measure our success by countermeasure deliverables, and not just scientific papers. By project, the mature (CRL 5-7) candidates are:
 - Short Radius, Intermittent Artificial Gravity (Young).
 - Preflight and Inflight Gait Adaptability Training (Bloomberg)
 - Locomotion Disturbance Assessment Techniques (Wall/Bloomberg)
 - Dynamic visual acuity tests (Bloomberg)
 - Intranasal Scopolamine (Putcha)
 - Preflight Orientation Training Methods (Oman)
 - Inflight disorientation reduction via improved standards for spacecraft interior visual cues, egress paths, and workstation layouts (Oman)

Some of these countermeasures already have JSC advocates, and the transition path is relatively clear. We will need to work with CEVP (Clarence Sams, Edna Fiedler) to develop transition plans for the others. In some cases, we expect it will be appropriate to form science teams to design additional ground control studies or pre/postflight or flight

Supplementary Medical Objectives (SMOs) in order to fully demonstrate countermeasure efficacy. Timeframe: Winter 04.

Concepts in earlier (CRL 2-4) stages of development include:

- Vibrotactile orientation displays (Wood, Wall)
- Teleoperator skill prediction techniques (Oman)
- Baclofen for motion sickness (Young)
- Large radius, continuous Artificial Gravity (Young)
- Postflight and Inflight Gaze Stability Assessment Techniques (Reschke)
- Unilateral otolith assessment tests (Dornhoffer/Wyuts)

(The Reschke and Dornhoffer/Wyuts developments are presently on hold, pending proposal resubmission, review and resources.)

2. Update the Team Strategic Plan based on NASA's maturing vision for space exploration, and the evolving Bioastronautics Roadmap. The most recent revision (2003) gave priority to long duration Earth orbital missions, and did not incorporate the new vision for exploration missions e.g. Moon and Mars. As part of this effort, the set of "Enabling Questions" (Table 3) also needs to be revised. Some of the questions are very general, and open ended. The questions should be defined in more answerable ways. Timeframe: December 04-January 05.
3. Participate in the development of the neurological function components of the NASA astronaut Clinical Status Evaluation (CSE). This program is particularly important since it offers the possibility of obtaining a consistent longitudinal dataset on the effects of spaceflight on all partner astronauts. Drs. Reschke, Clark, Oman, and Paloski will participate in an international workshop to be held in Houston, TX on December 8-10, 2004. Several NSBRI countermeasure concepts (above) will be among those considered. The goal of CSE is to utilize a set of well validated, practical, integrated protocols to evaluate postflight performance (e.g. emergency egress capability), fitness for return-to-duty, and efficacy of rehabilitation. There is no conclusive evidence that prolonged (months to years) exposure to 0-G produces irreversible vestibular changes, but vigilance is appropriate, since only half a dozen individuals have yet flown beyond 6-8 months. Anatomical changes have been seen in vestibular sensory epithelia in animals on flights of several weeks and longer, but the functional significance of these changes is unclear. Radiation exposure is known to impact some areas of the central vestibular system (e.g. hippocampus). The relative lack of validated, sensitive instrumentation and methods for early detection of impairment of vestibular function comparable to those available to assess auditory or visual function remains a continuing change.
4. Develop an international, interdisciplinary strategic plan for development and evaluation of Artificial Gravity as a countermeasure. Dr. Paloski is leading NASA's AG program, and a NASA plan is in the final stages of development. Individual and team research projects, followed by live-aboard ground studies and a flight research project on humans

and/or animals is forseen. Dr. Young also heads an IAF subcommittee on AG. This international group is writing a White Paper on use of Artificial Gravity. During the coming year, several members of Dr. Young's AG project are contributing to a NASA "Phase 1" AG/Bedrest evaluation study, and hope to subsequently participate in an International Multi-discipline Artificial Gravity (IMAG) project. Timeframe: 2004-2005

5. Increase out team's interaction with Flight Surgeons and Astronauts. After a period of relative openness to discussions of disorientation and motion sickness, we have recently noted a renewed reluctance to discuss neurovestibular symptoms among astronauts, and a lack of neurovestibular expertise among flight surgeons, all somewhat reminiscent of the pre-Skylab era. Our PIs should participate in Grand Rounds lectures, formal and informal astronaut debriefs. Also under consideration is development of an "Astronaut Neurovestibular Primer", a practical tutorial on known neurovestibular problems and solutions, written in lay language for busy astronauts, engineers, and NASA and Congressional decision makers, emphasizing lessons learned from the Shuttle/Spacelab and Mir programs. Timeframe: begin fall 2004.
6. Augment our team's interactions with other NSBRI research teams and with habitability experts in Space Human Factors Engineering. Areas of potential interaction include: 1) a interdisciplinary program in Pharmacotherapeutics organized by Dr. Putcha; 2) coordination of human performance assessment test protocols currently in use by our team (Young/Jarchow, Oman, Putcha/Buckey), Smart Medicine (Putcha/Buckey), and Neurobehavioral (Kosslyn); and 3) neurovestibular updates to the new revision of NASA Man Systems Integration Standard 3000, (working with Janis Connolly of JSC).
7. Promote interaction of team members with engineers and mission planners working on development of Exploration Missions, e.g. with teams funded under the NASA Exploration Systems Mission Directorate Broad Agency Announcement (BAA). For example, the MIT Draper Laboratory is leading an architectural trade study examining Lunar Mission Architectures and Crew Exploration Vehicle design concepts. In order to properly assess the sensory-motor adaptation risks on exploration missions, it is important to have a good understanding of the type and size of potential exploration mission vehicles, including possible module configurations, 0-G transit and surface times, the anticipated role of astronauts in supervisory and manual vehicle control in landing and docking, the requirements for 0-G and early postlanding EVAs on exploration missions, and the engineering issues which constrain artificial gravity systems. It is already clear that Moon and Mars missions will likely involve more g transitions and complex docking and EVA activity than on low Earth orbit missions, so sensory-motor adaptation issues may become even more important than in the past.
8. Fill gaps in the team's research portfolio through the annual research solicitation process. Areas identified in the current strategic plan include autonomic- emetic physiology, landing vertigo, and post-flight rehabilitation. NSBRI should not only continue to develop new anti-emetic drug delivery techniques, with an emphasis on drugs suitable for "rescue therapy", capable of stopping motion sickness "in its tracks".

but also track progress by NIH funded autonomic/emetic physiologists, perhaps through a NSBRI Workshop. Recent progress (e.g. role of neurokinins, substance P etc) may provide new countermeasure opportunities. At present, we lack a project formally examining the visual and manual control limitations of a 0-G adapted astronaut attempting a manual or semi-automated landing. NSBRI currently has little research underway on rehabilitation, though the recent appointment of Vincent Caiozzo as Associate Team Leader for Nutrition may help us build this area. Neurovestibular rehabilitation is a subspecialization of this area, and should not be researched in isolation. We would like to further promote the use of mathematical modeling and molecular methods in our research, where appropriate.

9. Plan the next NSBRI Neurovestibular Adaptation Research special issue of the Journal of Vestibular Research and an International Space Neurovestibular Symposium. A committee consisting of Drs. Paloski (NASA), Oman (NSBRI), Young (NSBRI) and Black (USRA) has been formed to organize the Seventh Symposium on the Role of the Vestibular Organs in Space Exploration, continuing the tradition of these symposia initiated by Dr. Ashton Graybiel in the 1960s, and most recently held in Portland in September 2002. The current plan is to hold a three day meeting in Europe in 2006, which we hope will make it easier for Russian and European scientists, flight surgeons, and astronauts/cosmonauts to participate. As in prior years, we hope to obtain sponsorship to partially offset meeting costs and support student participation from NASA, NSBRI, NIH, and industry, and will also approach ESA, DLR and CNES.
10. Participate in the NSBRI graduate and postdoctoral education programs. Several members of our team are participating in phase 1 graduate program development activities underway both at JSC and in the Harvard-MIT Division of Health Sciences. We should also recruit applicants for the NSBRI postdoctoral fellowship program.
11. Update the Neurovestibular Adaptation Team portion of the NSBRI web site. The existing NSBRI site consists of relatively terse project descriptions. Interesting photos and data examples have been collected and could be added to the NSBRI site or a subsidiary site. Photos illustrating relationship to clinical neuro-otology and associated impacts on America could also be included.

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I. ABSTRACT

Optimal human performance during space exploration requires the maintenance of all physiological systems, which in turn is dependent on adequate nutrition and physical fitness. The primary critical issues being addressed by the nutrition and physical fitness team are loss of muscle mass and muscle strength; and radiation-enhanced cancer. The nutrition and physical fitness program became operational in 2001. It presently consists of four countermeasure projects (Lupton, Wolfe, Ferrando, and Roubenoff). The Ferrando project is just beginning and the Tobin project, funded three years ago is now being completed. The original cornerstone project is the Wolfe bed rest study, which has seven "add on" projects and is fully integrated with the Tobin and Roubenoff projects. A critical finding from the Wolfe bed rest study was that nutrition may ameliorate muscle wasting but it does not preserve the loss of muscle strength. This reinforced the need for a combined nutrition/physical fitness intervention strategy, which is now the subject of both the Roubenoff and the newly funded Ferrando project. The Lupton project on the development of nutritional countermeasures to radiation enhanced colon cancer resulted in several discoveries: that exposure to 1 Gy iron ions does, in fact, enhance preneoplastic markers of colon tumorigenesis, and that fish oil feeding and a fiber supplement can ameliorate this negative effect. Importantly, if rats are provided diets high in both fish oil and the fermentable fiber pectin, the enhanced tumorigenic effect seen with radiation exposure is no longer observed. At this time, the nutrition and physical fitness team considers that it has five potential deliverables. They are: (1) A nutrition supplement to protect against radiation-enhanced colon cancer. (2) A nutrition supplement to protect against muscle wasting. (3) A resistance exercise protocol to protect against loss of muscle mass and strength. (4) A protocol for the timing of supplement intake to exercise. (5) A noninvasive technology to monitor changes in colon gene expression profiles over time. In summary, the nutrition and physical fitness team addressed two key critical risks during the 2004 fiscal year: loss of muscle mass and strength and radiation-enhanced carcinogenesis. Three projects, highly integrated with each other, focused on countermeasures against muscle loss and strength, and one project (coordinated with NASA's radiation program) focused on a nutritional countermeasure against radiation-enhanced colon cancer. The Nutrition and Physical Fitness team has fully integrated its existing projects, expanded by the addition of small projects, and a new bed rest study which incorporates both nutrition and physical fitness. Optimal diet and physical activity protocols for space flight will impact every aspect of astronaut health and performance.

II. INTRODUCTION

The team's organization and activities, and risk areas addressed. The Nutrition and Physical Fitness Team became operational in 2001. It presently consists of four projects (Lupton, Wolfe, Ferrando, and Roubenoff), however during fiscal year 2004 the Tobin project was being completed and the Ferrando project had not yet started. Thus, this report covers Tobin but not Ferrando, except when future plans are discussed. The Lupton, Wolfe and Tobin projects are directed to nutrition countermeasures and the Roubenoff project combines both nutrition and physical fitness. The two major problems encountered in space that are currently addressed by the Nutrition and Physical Fitness Research Program are (1) muscle alterations and atrophy and (2) radiation enhanced development of cancer. With respect to muscle alterations and atrophy, the following are the relevant risks (numbered in parentheses), found on the Critical Path Roadmap, that may be ameliorated by nutrition and physical activity interventions: skeletal muscle atrophy (13); increased susceptibility to muscle damage (14); inadequate nutrition (18); accelerated bone loss and fracture risk (1). In addition to the relevant risks addressed by the Wolfe project, the Roubenoff project addresses allergies and autoimmune diseases (12) and immunodeficiency/ infection (8).

Both appropriate nutrition and physical fitness can have a significant impact on muscle mass and strength. Nutrition is required to provide amino acids for muscle protein synthesis and energy for strength and endurance. Muscle protein synthesis is known to be depressed during space flight, due, in large part to muscular inactivity. This depression of protein synthesis is accompanied by an increase in protein degradation due to a moderate level of hypercortisolemia observed during space flight. Both aerobic and resistive exercise are also critical for proper muscle function. Aerobic exercise helps maintain blood flow to muscle so that nutrients can reach myocytes. Resistive exercise is key to maintaining muscle strength. Not only must energy expended on physical activity be balanced by appropriate food intake, the timing of exercise with respect to food ingestion impacts such important physiological effects as uptake of amino acids into muscle. Thus specific nutrients, ingested at the appropriate time, may help to maintain muscle mass.

With respect to radiation enhanced development of cancer: The risks to personnel in space from naturally occurring radiations are generally considered to be the most serious limitation to human space missions and research in this area is now a top priority for NASA. Ionizing radiation results in the production of reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and hydroxyl radical, which are mutagenic and well documented to be carcinogenic in animals and humans. ROS accumulate with time, and it has been shown in a number of different systems that the greater the production of ROS, the higher the level of oxidative DNA damage. ROS can permanently damage nucleic acids inducing some 20 major oxidative DNA adducts some of which can go on to form tumors. Interestingly, diet may play an important role in removal of these DNA-adducted cells and thus protect against radiation-enhanced tumorigenesis. The NSBRI funded project of Lupton et al. uses diet as a countermeasure to selectively remove DNA-damaged cells from the colon by targeted apoptosis. Colon cancer is chosen for the model system as it is the second leading cause of death from cancer in the United States today, it strikes men and women equally, and it is the cancer most responsive to diet. The relevant risks on the critical path that are addressed are: radiation-enhanced carcinogenesis (31) and inadequate nutrition (18).

Specifically, *Nutritional Countermeasures to Radiation Exposure*, JR Lupton, PI, Texas A&M University, is testing the hypothesis that a particular diet intervention (an n-3 lipid and fermentable fiber combination) in rats should protect against radiation-enhanced colon cancer by targeting DNA damaged cells for apoptotic removal. Rats receive one of four diets, are exposed to heavy iron radiation at Brookhaven National Laboratory and are injected (or not) with a colon specific carcinogen. A variety of measurements are taken at three stages of the tumorigenic process (initiation, promotion, and final tumor development). This project also has a noninvasive component of monitoring changes in gene expression over time as a result of radiation and carcinogen exposure using microarray technology. If validated in rats, the diets and techniques can be modified for future studies in humans.

Skeletal Muscle Response to Bed Rest and Cortisol Induced Stress, R. R. Wolfe, PI, University of Texas Medical Branch at Galveston, is testing an amino acid supplement designed to ameliorate muscle wasting induced by stress-and microgravity-induced depression of protein synthesis in a bed rest study. The study consists of 12 individuals with or without consumption of the supplement in a 30-day bed rest trial. A unique feature of this study is the use of a cortisol infusion at two times during the intervention period to mimic (in part) the documented elevated cortisol levels during space flight. This study has formed the cornerstone of our research program and we have added a large number of ancillary grants which use the bed rest model and the nutritional intervention to address issues related to other goals.

Nutritional Modulation of Pancreatic Endocrine Function in Microgravity, B. W. Tobin, PI, Mercer University School of Medicine, is determining amino acid countermeasure effects on endocrine function of human pancreatic islets of Langerhans with the goal to optimizing insulin synthesis and secretion under microgravity conditions. Dr. Tobin uses human pancreatic islet cells cultured on static plates or in a high aspect ratio vessel (HARV) designed to replicate some of the conditions of microgravity. The goal of this research project is to determine how different physiological conditions, characterized by over or underexpression of certain hormones, affect insulin secretion and to develop an amino acid combination that will optimize this secretion. Optimal insulin synthesis, should maximize uptake of amino acids into muscle and thus enhance muscle protein synthesis. Tobin's project is using the amino acid levels found in blood of subjects from Wolfe's bed rest study who have received his amino acid supplement. Thus, there is integration between these two projects.

Timed Feeding and Resistance Training To Prevent Muscle Atrophy, Ronenn Roubenoff, M.D. PI, Tufts University combines both nutrition and physical fitness in addition to the timing between the two. Our team has as its first priority combining nutrition and physical fitness to ameliorate muscle wasting. This project which was funded early in fiscal 2004, is proceeding on time and six of the subjects have completed the bed rest portion of the study. The project has the same experimental design and the same amino acid supplement as the Wolfe bed rest study so that results can be compared. The amino acid supplement is combined with an exercise component and a second variable is the timing of the supplement intake with respect to exercise. This project in combination with the Wolfe project should provide definitive (ground based) answers to whether exercise when added to the nutritional supplement at the right time can ameliorate muscle loss and the diminution of muscle strength. This research program serves to integrate both nutrition and physical fitness.

Relationships among the projects and with other teams. The Wolfe, Tobin, and Roubenoff projects are fully integrated with each other. For example, the Wolfe and Roubenoff bed rest

studies have the same inclusion and exclusion criteria for subjects and the same bed rest protocols. They test the same amino acid supplement, however the Roubenoff project adds both an exercise component and a timing intervention of the supplement with respect to the exercise intervention. The Tobin project uses the blood levels of amino acids achieved in the Wolfe project subjects as the amino acid mixture presented to the human pancreatic islet cells. Tobin has "loaned" a co-investigator from his laboratory, who is an expert in immune response, to the Wolfe study in Galveston to measure immune response to the amino acid supplement in the bed rest study so that it can be mimicked in the in vitro pancreatic islet cell studies.

The Lupton project is integrated with NASA's radiation effort. She has received a separate NASA grant during this time period to further develop one key aspect of the NSBRI grant. The non-invasive methodology to detect changes in colon cell gene expression patterns over time as a function of radiation exposure and diet treatment is being optimized for use in humans. In addition, a member of the bone team, Stefan Judex, is using bones from the irradiated rats to determine the effect of the nutrition countermeasure on bone loss.

In summary, the nutrition and physical fitness team addressed two key critical risks during the 2004 fiscal year: loss of muscle mass and strength and radiation-enhanced carcinogenesis. Three projects, highly integrated with each other, focused on countermeasures against muscle loss and strength, and one project (coordinated with NASA's radiation program) focused on a nutritional countermeasure against radiation-enhanced colon cancer.

III. TEAM ACCOMPLISHMENTS (October 1, 2003-September 30, 2004).

Advances toward and testing in flight. At this time, the nutrition and physical fitness team considers that it has five potential deliverables. They are: (1) A nutrition supplement to protect against radiation-enhanced colon cancer. (2) A nutrition supplement to protect against muscle wasting. (3) A resistance exercise protocol to protect against loss of muscle mass and strength. (4) A protocol for the timing of supplement intake to exercise. (5) A noninvasive technology to monitor changes in colon gene expression profiles over time. The nutrition supplement to protect against radiation-enhance colon cancer and the nutrition supplement to protect against muscle wasting will be discussed in detail below. The resistance exercise protocol to protect against loss of muscle mass and strength is part of the Roubenoff project which is in its first year and thus has no data to present at this time. Also, a new project from A. Ferrando (which is just starting now) will be testing an exercise protocol with the amino acid supplement. Roubenoff is also addressing the timing of exercise with respect to supplement intake. Finally, the Lupton project competitive renewal will optimize noninvasive technology to monitor changes in colon gene expression profiles over time.

A nutrition supplement to protect against radiation-enhanced colon cancer. The Lupton project, Nutritional Countermeasures to Radiation-Enhanced Colon Cancer, was completed September 30, 2004, and the competitive renewal began October 1, 2004 and will thus be part of next year's annual report. Full length manuscripts resulting from this grant to date include:

Turner ND, Braby LA, Ford J and Lupton JR. Opportunities for nutritional amelioration of radiation-induced cellular damage. Nutrition 18:904-912, 2002.

Hong MY, Chapkin RS, Barhoumi R, Burghardt RC, Turner ND, Henderson CE, Sanders LM, Fan YY, Davidson LA, Murphy ME, Spinka CM, Carroll RJ and Lupton JR. Fish oil

feeding increases the unsaturation index in mitochondrial phospholipids, enhancing reactive oxygen species generation and initiating apoptosis in rat colonocytes. *Carcinogenesis* 23:1919-1925, 2002.

Hong MY, Chapkin RS, Davidson LA, Turner ND, Morris JS, Carroll RJ, and Lupton JR. Fish oil enhances targeted apoptosis during colon tumor initiation in part by down regulating bcl-2. *Nutr Cancer* 46:44-51, 2003.

*Apanasovich TV, Lupton JR, Popovic N, Chapkin RS, Turner ND, Sheather S, Carroll RJ. Testing for spatial correlation in binary data with application to aberrant crypt foci in colon carcinogenesis. *Biometrics* 59:752-761, 2003.

*Wu G, Fang Y-Z, Yang S, Lupton JR, and Turner ND. Glutathione metabolism and its implications for health. *J. Nutr.* 134: 489-492, 2004

*Sanders LM, Henderson CE, Hong MY, Barhoumi R, Burghardt RC, Carroll RJ, Turner ND, Chapkin RS, and Lupton JR. Pro-oxidant environment of the colon compared to the small intestine may contribute to greater cancer susceptibility. *Cancer Lett.* 208:155-161, 2004.

Ones marked with an asterisk are from the period of fiscal year 2004. This research program tests the efficacy of a nutritional supplement (fish oil, high in omega 3 fatty acids and pectin, a highly fermentable fiber) to protect against radiation-enhanced colon cancer. The research has shown that at each stage of the tumorigenic process (initiation, promotion, and final tumor development) fish oil is protective against the appropriate marker of tumorigenesis – DNA damage at the initiation stage, aberrant crypt formation at the promotion stage, and tumors at the final tumor stage. Selective data are shown below.

Initiation stage of colon carcinogenesis. We have shown that the fish oil/pectin fed rats have lower levels of oxidative DNA adducts in colonocytes than rats fed corn oil/cellulose. Thirty rats per diet group were provided with either corn oil or fish oil containing diets and oxidative DNA adducts (8-OHdeoxyguanosine) were measured immunohistochemically in colon crypts. The fish oil fed rats had lower levels of DNA damage than did the corn oil fed rats (Figure 1).

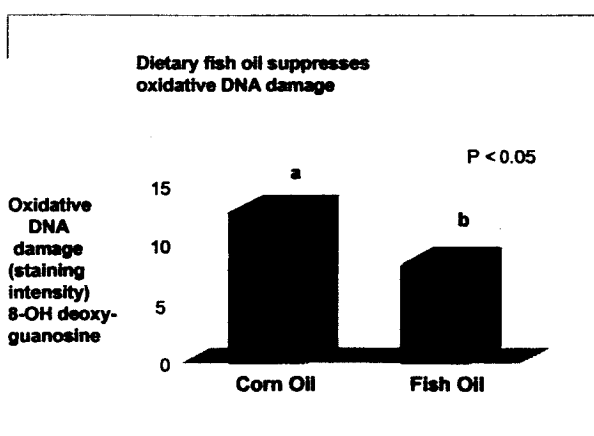


Figure 1. Fish oil fed rats had lower levels of DNA damage in colonic crypts than did corn oil fed rats.

In addition, we have shown that dietary fish oil and pectin are protective during this initiation stage of colon cancer by creating a pro-oxidant environment that enhances apoptosis. We hypothesized this dietary combination will also protect against radiation-enhanced colon carcinogenesis via redox modulation. To test this, 60 SD rats were fed one of four diets (15% fish or corn oil, 6% pectin or cellulose) for 3 wk prior to 1 Gy of 1 GeV Fe ion irradiation followed by injection with the colon carcinogen azoxymethane (AOM) or saline 10 d later. Glutathione (GSH) and oxidized glutathione (GSSG), markers of redox status, were examined in colonic mucosa by HPLC. Prior to AOM exposure, irradiated rats fed fish oil/pectin exhibited a more reduced colonic environment (Fig. 2B) than corn oil/cellulose fed rats (Fig 2A) ($\downarrow$ GSSG ($p < 0.05$), $\uparrow$ GSH/GSSG). Upon subsequent exposure to AOM, GSH increased in all diets ($p < 0.05$). Yet, between 6 and 12 h post-AOM, fish oil/pectin fed rats exhibited an oxidative shift ($\downarrow$ GSH, GSH/GSSG ($p \leq 0.05$)) almost twice that seen in the corn oil/cellulose diet (Fig. 2C). In fact, GSH continued to increase in corn oil/cellulose fed rats until 9 h post AOM ($p < 0.05$) and an oxidative shift did not occur until 24 h post AOM ($p < 0.05$) (Fig. 2C). These data suggest the ability of the fish oil/pectin diet to create a more reduced colonic environment in response to radiation may enable the colon to make a substantial oxidative shift, an important signal for apoptosis, when confronted with another insult, such as a chemical carcinogen.

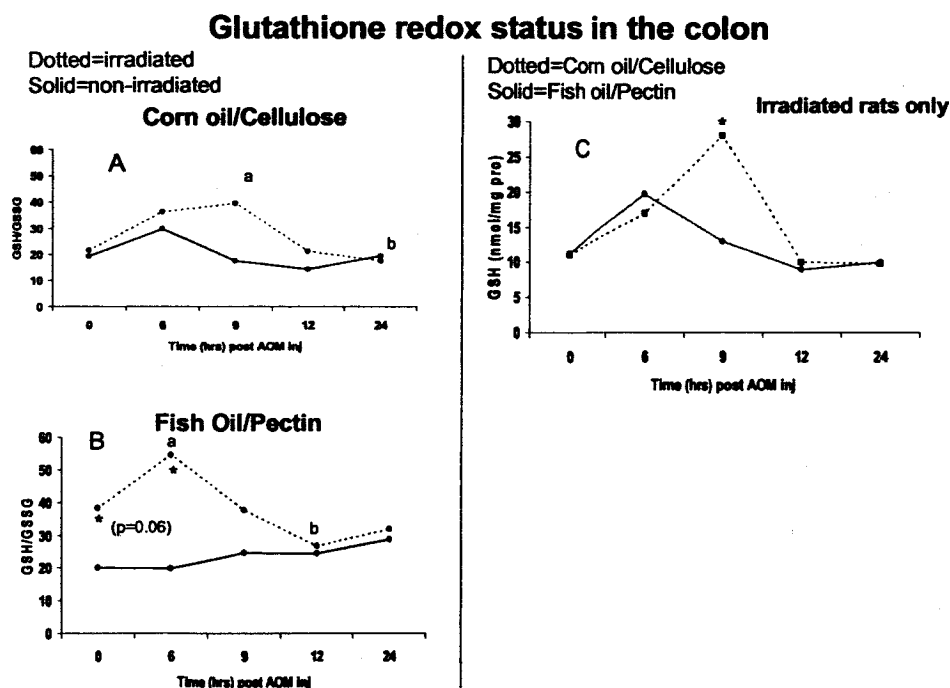


Figure 2. Panel A shows changes in the GSH/GSSG ratio over time in corn oil/cellulose fed rats. Dotted lines are the irradiated rats and solid lines are the non-irradiated rats. Panel B shows the same information as Panel A, but for the fish oil/pectin fed rats. Panel C shows irradiated rats only. Dotted lines represent the corn oil/cellulose rats, and the solid black line represents the fish oil/pectin rats.

Promotion Stage of Colon Tumorigenesis. To determine the effect of diet on the promotion stage of colon tumorigenesis, 80 rats (40 irradiated, and 40 non-irradiated) were provided one of

four diets in a 2 x 2 factorial design (fish oil/corn oil) (cellulose/pectin). The irradiated rats received 1 Gy iron after receiving their experimental diets for 3 weeks. All rats were injected with azoxymethane twice at 10 and 17 days post irradiation. Rats were terminated 6 weeks post the second AOM injection. Irradiated rats had a significantly greater number of high multiplicity aberrant crypts than did non-irradiated rats (Figure 3). Fish oil reduced the number of high multiplicity aberrant crypts compared to corn oil (Figure 4). Pectin resulted in an increased apoptotic index in colonocytes compared to corn oil (Figure 5).

Results – ACF

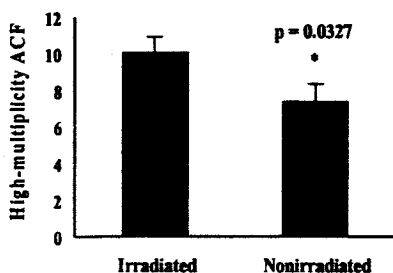


Figure 3. Irradiated rats had higher levels of high multiplicity aberrant crypts than did non-irradiated rats

Results – ACF

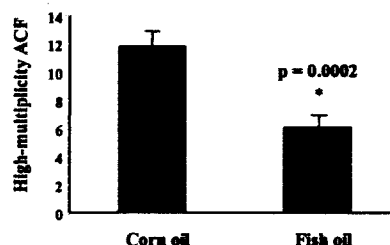


Figure 4. Fish oil fed rats had lower levels of high multiplicity aberrant crypts than did corn oil fed rats

Results – Apoptotic Index

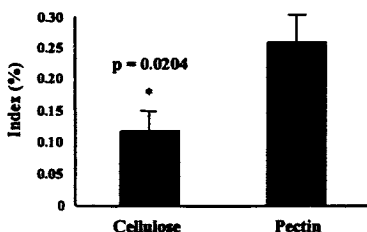
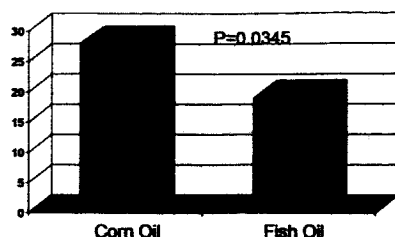


Figure 5. Pectin feeding resulted in a greater apoptotic index than did feeding cellulose.

Final Tumor Stage. Two separate analyses were run: using data from all animals used in the study and the animals surviving to the end of study, 31 week after the 2nd AOM injection. Using the data from all the animals used in the study, cumulative tumor incidence graph was plotted as a function of time (any types of tumors at any location were considered). It was found that irradiated rats started developing tumors earlier than non-irradiated ones. The effects of oil, fiber, radiation treatment and their possible combinations were then analyzed. Oil was found to be a significant factor predicting the probability of having a colon tumor ($P=0.0345$ and $P=0.0202$ when all animals and only animals surviving to the end of the study were considered, respectively). All rats fed with corn oil based diets were found to have 1.98 times higher colon tumor incidence than rats fed with fish oil based diets, and the chances of having tumors for corn oil based diet group representatives increased to 2.2 times for the rats killed at the end of study ($P=0.0345$ and $P=0.0202$ respectively).

Proportion of rats with colon tumors



Collectively these data support using a supplement of fish oil and pectin to protect against radiation-enhanced colon carcinogenesis. We are comfortable with recommending a supplement of omega 3 fatty acids and pectin as a supplement for testing in astronauts in a flight study for the following reasons: Our data are supportive of a beneficial effect at each stage of the colon tumorigenic process; fish oil (containing eicosapentaenoic acid (EPA) and docosahexaenoic acid) (DHA) has been shown to be protective against coronary heart disease. There is now a recommendation from the US Government to eat two fish meals per week (from the Dietary Guidelines Advisory Committee that the team leader, Dr. Lupton served on) because of the EPA and DHA found in certain fish. Also, there is now a Dietary Reference Intake Value (DRI) for fiber as a result of the National Academy DRI report on the macronutrient (a committee that Dr. Lupton chaired). Since Americans are being encouraged to increase their intake of both n-3 fatty acids and dietary fiber, for a multitude of health benefits, it would seem appropriate to test the effects of these supplements of the combination of fiber and fish oil to also protect against radiation-enhanced colon cancer.

A nutrition supplement to protect against loss of muscle mass and muscle strength. The Wolfe project, Skeletal Muscle Response to Bed Rest and Cortisol Induced Stress, was completed September 30, 2004, and the competitive renewal began October 1, 2004 and will thus be part of next year's annual report. Full length manuscripts resulting from this grant during the last year include:

- Paddon-Jones, D., Sheffield-Moore, M., Urban, R.J., Aarsland, A., Wolfe, R.R. Ferrando, A.A. The catabolic effects of prolonged inactivity and acute hypercortisolemia are offset by dietary supplementation. *J. Clin. Endocrinol Metab.* (in press)
- Paddon-Jones, D., Sheffield-Moore, M., Aarsland, A., Wolfe, R.R., Ferrando, A.A. Amino acid supplementation stimulates muscle anabolism while preserving response to a meal. *Am. J. Physiol. Endocrinol. Metab* (in press)
- Paddon-Jones, D., Wolfe, R.R., and Ferrando, A.A. Amino acid supplementation for reversing bedrest and steroid myopathies. *J. Nutr.* (in press).
- Paddon-Jones, D., Sheffield-Moore, M., Urban, R.J., Sanford, A.P., Aarsland, A., Wolfe, R.R. Ferrando, A.A. Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss during 28 days bedrest. *J. Clin. Endocrinol Metab.* Sept. 2004
- Paddon-Jones, D., Sheffield-Moore, M., Zhang X-J, Volpi, E., Wolf, S.E., Aarsland, A., Ferrando, A.A. and Wolfe, R.R. Amino acid ingestion improves muscle protein synthesis in the young and elderly. *Am. J. Physiol. Endocrinol. Metab.* Oct 28. 2003



Subject in Wolfe Bedrest Study

Most importantly we have chosen the bed rest study as the cornerstone of our program, and as such have requested and received funding for a number of important additional projects which use samples from this study. The following are descriptions of "add ons" to the Wolfe bed rest study. Data collection is complete from all of these studies. A summary of the results of each of these studies follows.

1. Helen Lane, Ph.D.

NASA Johnson Space Center
Houston, TX

Title: Response of oxidative capacity in skeletal muscle to prolonged bed rest.

The NSBRI project evaluated a nutritional countermeasure, protein/amino acid supplementation, on leg muscle performance after a month of bed rest. She showed that the ability to regenerate muscle ATP during exercise is blunted during bed rest.

2. Scott M. Smith, Ph.D. (Also a collaborator on the Tobin project)

Research Nutritionist
NASA Johnson Space Center
Houston, TX

Title: Markers of bone and calcium metabolism

Dr. Scott M. Smith's supplement to the Wolfe project is designed to measure markers of bone and calcium metabolism in the bed rest subjects. These include serum osteocalcin and bone-specific alkaline phosphatase, and urinary collagen crosslinks. Osteocalcin and Bone-Specific Alkaline Phosphatase are both bone formation markers. The collagen crosslinks are bone resorption markers, and represent a family of compounds which include n-telopeptide, pyridinoline, and deoxypyridinoline, among others. This study showed that calcium and bone resorption markers were both increased in urine as a function of the amino acid supplement. This was important information as we do not want to increase muscle strength and muscle mass but also increase bone loss. We are following up on this analysis in the current bedrest study from this group.

3. Brian W. Tobin, Ph.D.

Associate Professor
Mercer University School of Medicine
Macon, GA

Title: Blood amino acid levels from Wolfe project for use in Tobin project

In order to test the same protocols in several model systems, the amino acid blood levels produced by the Wolfe supplement have been quantitated and will be used as one of the interventions in the Tobin protocol. This is at no added expense to NSBRI.

4. Peter N. Uchakin, Ph.D. (Also a lead investigator on the Tobin project)

Lead Research Scientist

Division of Basic Medical Sciences

Mercer University School of Medicine

Macon, Georgia

Title: The effect of bed rest and corticosteroid treatment on the secretion of pro- and anti-inflammatory cytokines

Dr. Peter N. Uchakin is investigating the effects of bed-rest and corticosteroid treatment on the secretion of pro- and anti-inflammatory cytokines such as IL-1, IL-6 and IL-10. In addition, Dr. Uchakin will assess immunocyte distribution in whole blood. He found that as a function of bedrest and cortisol administration, the balance between the two classes of cytokines shifted towards humoral from cellular immunity and this was associated with Epstein barr virus reactivation.

5. Robert Fitts, Ph.D.

Marquette University

Milwaukee, WI

Title: Single muscle fiber function in response to bed rest and nutritional intervention.

Investigation of the contractile properties and force of single muscle fibers of the soleus (predominantly Type I) and vastus lateralis (predominantly mixed) in response to prolonged bed rest with and without the nutritional intervention. He found that an amino acid supplement protected against type II fiber peak velocity decline.

6. T.P. Stein, Ph.D.

Professor of Surgery

UMDNJ-SOM

Stratford, NJ

Title: Markers of oxidative stress

Oxidative damage from free radicals to DNA and lipids has been implicated in the etiology of a wide variety of chronic diseases and acute pathological states. Dr. Stein and his colleagues had the opportunity to obtain data on the question of whether space flight has any effect on the oxidative status of astronauts. They measured the urinary excretion of 8-iso-PGF2a and 8-oxo-7,8 dihydro-2 deoxyguanosine (8-OH dG) on 6 subjects (2 US astronauts and 4 Russian cosmonauts) before, during and after long duration space flight on the Russian space station MIR. The urinary excretion of the isoprostane 8-iso-PGF2a and 8-OH dG are markers for oxidative damage to lipids and DNA respectively.

In summary, the Wolfe/Ferrando bed rest study intervention phase was completed ahead of schedule and data from the ancillary projects are now being prepared for publication. The major findings are that bedrest, per se, has a negative effect on protein synthesis, muscle mass, muscle strength, type I and II muscle fiber peak power, ATP recovery from a bout of resistance exercise, cellular immune function and reactivation of a latent virus. Cortisol administration exacerbates these negative effects. An amino acid supplement mitigates a large number of these negative outcomes but does not eliminate them.

Timed Feeding and Resistance Training To Prevent Muscle Atrophy, Ronenn Roubenoff, M.D. PI, Tufts University combines both nutrition and physical fitness in addition to the timing between the two. This study is fully integrated with the Wolfe bedrest study and will also

determine the combined effect of the amino acid supplement and resistive training, and the timing of the supplement with respect to the exercise to maximize the effectiveness of both.

Nutritional Modulation of Pancreatic Endocrine Function in Microgravity, B. W. Tobin, PI, Mercer University School of Medicine, is determining amino acid countermeasure effects on endocrine function of human pancreatic islets of Langerhans with the goal to optimizing insulin synthesis and secretion under microgravity conditions. He has optimized the incubation protocol so that it has now been adopted by centers doing pancreatic islet transplants. Results from his studies show that pancreatic cells exposed to simulated microgravity conditions have a lower need for glucose, and a greater utilization of cysteine and phospholipids than their traditionally cultured counterparts.

IV. TEAM PLANS

For the next 12 months our plans are to: complete publication of the manuscript from the Lupton and Wolfe studies; complete three quarters of the subjects in the Roubenoff bedrest project and half of the subjects in the newly funded Ferrando project. During this coming year we will also work towards maximizing the potential for two countermeasure deliverables: the fish oil/fiber supplement for radiation enhanced cancer and the amino acid supplement as a countermeasure against muscle wasting and loss of strength. Progress in each of these areas is discussed above. Our eventual goal with the current projects we now have on our team is to deliver five countermeasures at the end of the funding period (2008). Those countermeasures are (1) A nutrition supplement to protect against radiation-enhanced colon cancer. (2) A nutrition supplement to protect against muscle wasting. (3) A resistance exercise protocol to protect against loss of muscle mass and strength. (4) A protocol for the timing of supplement intake to exercise. (5) A noninvasive technology to monitor changes in colon gene expression profiles over time. Since countermeasures (1) and (2) have been discussed in some detail in Section III, a brief description of the countermeasures 3-5 will be discussed in this section.

Development of an exercise protocol, and the appropriate equipment to maximize both muscle strength, lean body mass, bone strength, and aerobic capacity is key to overall performance in space. Both the Ferrando study and the Roubenoff study will deliver the impact of resistance exercise on a wide variety of parameters. Since exercise takes time from other tasks and also requires energy input, which means greater food intake, maximizing the effectiveness of an exercise program will have many benefits. In addition, the Nutrition and Physical Fitness Team is aware that different forms of preflight and in-flight physical exercise are a major countermeasure thrust for the Muscle Team and will work with the Muscle Team to coordinate and maximize the effectiveness of our collective programs to address shared goals.

Development of a strategy of timing of food intake with respect to physical activity has been overlooked to date. This countermeasure plan will be key to the overall health of individuals in space. When one eats with respect to when one exercises has important consequences for overall utilization of nutrients and for human performance. The current recommendations for food intake timing with respect to exercise as practiced in flight are not based on strong scientific studies. A scientific basis for the timing of food intake and exercise prescriptions is needed. For example, R. Wolfe has shown in human studies, that providing amino acids prior to rather than after an exercise bout will enhance protein synthesis by up to three fold. The appropriate combination of

foods or new functional foods with time release components could provide a certain level of blood glucose over extended periods of time so that exercise or other tasks such as EVA could be performed without stopping to eat. The current Roubenoff project should help resolve some of the issues with respect to timing of food intake with respect to exercise.

Finally, the Lupton project which was just funded to begin October 1 will develop noninvasive technologies for assessing the effectiveness of diet interventions. One important aspect of this project is the use of microarray technology on mRNA from fecal material to see which genes are turned on or off during particular diet interventions, which ones are affected by radiation exposure and how these gene array patterns predict for a variety of endpoints. This patented technique is well developed in the rat, and the plan is to later apply it to humans.

Each of these projects has important earth-based applications. For example, a protein supplement that will enhance amino acid uptake into muscle and muscle protein synthesis can be used for burn patients (and Wolfe is already testing this supplement on that population). It will also be useful for individuals on earth who have muscle wasting due to a variety of causes. The fish oil/pectin supplement will also be useful in earth-based applications. We have already shown that this combination is protective against chemical carcinogen damage in addition to radiation damage. With colon cancer the second leading cause of death from cancer in the US today, such a supplement could prove to be very beneficial.

Finally, a major goal of our team for the coming year is to increase our collaborative efforts with other teams, particularly the muscle and bone teams. In addition to strong collaborations within our team, a few examples of integration of the Nutrition and Physical Fitness Team with other teams or researchers outside of the NSBRI are as follows: Lupton is collaborating with Judex (bone team) in supplying rat hind limbs from irradiated rats on different diets. The Wolfe bed rest study is a true collaborative effort with the following investigators/projects: P. Uchakin, Mercer University, testing the hypothesis that stress during inactivity alters the balance between cell-mediated and humoral immunity; S. M. Smith, NASA, JSC, The effect of bed rest and amino acid supplementation on bone markers of calcium metabolism; R.R. Fitts, Marquette University, The effect of prolonged bed rest and amino acid supplementation on muscle fiber function; R Stowe, UTMB, Effects of prolonged bed rest on herpesvirus-specific immunity. Similar measurements to the Stowe bed rest study are also being performed on the Shuttle and ISS crewmembers and so the Stowe study would serve to complement in-flight work. Additional collaborative projects with the Wolfe study include: T.P. Stein, UMD-NJ, Does bed rest + hypercortisolemia lead to increased oxidative stress during the recovery phase?; H W Lane, NASA, JSC, The effect of bed rest and amino acid supplementation on muscle energy production during exercise. In addition to collaborative projects, several people who were not directly funded with NSBRI grants have become an active part of our team. They include Helen Lane and Scott Smith from NASA/JSC in the area of nutrition, Don Hagan, also NASA/JSC for physical fitness, and Michelle Perchonok, NASA/JSC for food science. These close ties to NASA enable the Nutrition and Physical Fitness Team to be up to date on the most recent countermeasure approaches to addressing nutrition and physical fitness related risks.



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TEAM PROJECTS AND PRINCIPAL INVESTIGATORS

Guided High Intensity Focused Ultrasound (HIFU) for Mission-Critical Care

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I. ABSTRACT

The mission of the Smart Medical Systems Team (SMS Team) is to develop medical systems, including both technology and clinical approaches, that would address the critical medical risks that astronauts face in the special environment of space. Although this special space environment has its own specific risks, our principal concern is the limited medical technology, and restrictions on the use of this technology, that is available in the space environment. Accordingly, we seek to develop systems that can provide a high level of medical care, that are simple to use, lightweight and portable, and can be utilized by astronauts with limited training. Since the new paradigm for critical medical care is now "stand and fight", rather than "stabilize and return", the long range goal for this Team is to develop a set of autonomous medical care devices that would be used to meet many medical needs in outside LEO missions in which two-way communication is limited. These goals are particularly relevant to the Vision for Space Exploration.

At the present time, there are four active projects supported by the NSBRI in the SMS Team, with two additional projects that are supported by NASA that have a direct connection with SMS. All four projects involve some form of sensor-actuator model (in medical language, perhaps it would best be labeled diagnosis/therapy). This model presumes that one component of the system will diagnose a potential medical problem, and a second component of the same (or related) system will offer a countermeasure to this problem. For example, the project led by Dr. Lawrence Crum, from the University of Washington, intends to develop an image-guided therapy system in which a portable ultrasound scanner would be used to diagnose a condition—say internal bleeding—and then the same system would apply High Intensity Focused Ultrasound (HIFU) to induce hemostasis.

The projects headed by Dr. Scott Dulchavsky (Henry Ford Hospital; Wayne State University School of Medicine) and Dr. James Thomas (The Cleveland Clinic Foundation) would also use ultrasound for diagnosis; however, Dr. Dulchavsky is interested in developing mini-laparoscopy instruments that could be used in a microgravity environment to perform surgery, while Dr. Thomas would use echocardiography in his diagnosis and probably some form of drug for therapy; Dr. Thomas' project is jointly supervised with the Cardiovascular Alterations Team of the NSBRI. Dr. Dulchavsky also has a related project, supported directly by NASA, in which he has developed software that is currently being used in ISS Increments 9 and 10 to train astronauts in the use of the ultrasound device presently on the ISS. The fourth investigator in SMS, Dr. Babs Soller, (University of Massachusetts Medical School) has developed a non-invasive device that uses Near-Infrared Spectroscopy (NIRS) to measure blood and tissue physiological parameters. She has extended the application of this versatile device to measure the progress of exercise—a project that is supported by the NSBRI/NASA Space Medicine Program.

Finally, the SMS Team works very closely with Dr. Harold Doerr, from the Baylor College of Medicine in his project to develop and test a space-adapted human patient simulator. All of the projects fit within the strategic plan of the SMS Team for NSBRI countermeasure (CM) development (sections IIIC and IIID).

II. INTRODUCTION

A. Team Objectives

The SMS Team aims to take a leadership role in the research and development of advanced, integrated, and autonomous systems for astronaut health assessment, maintenance and medical care. This includes the delivery and evaluation of medical interventions and other CMs that reduce the deleterious effects of space travel and enhance the overall well being of astronauts. In achieving this goal, it is anticipated that there will be significant impact and applications for earth-based health and medical care. Early progress of team members also show a significant potential for technology transfer to the commercial sector, with two companies recently formed, and negotiations underway to start a third. A major sub-objective of this Team is to develop Commercial Off-the-Shelf (COTS) technology that would could be utilized by NASA to treat problems in space medicine.

B. Health Concerns and Hazards

Health problems associated with space travel may be related to the effects of microgravity, radiation and other risks to the body that are particular to space flight, but they may also be independent of these effects. Medical problems may arise in association with a given demographic population or as a result of a toxic environmental exposure. Moreover, complex interactions may result in alterations and disorders presenting and/or responding differently in a microgravity environment relative to earth. The unique medical circumstances, requirements and limited health care resources in space pose challenges and opportunities for new strategies of physiological monitoring, medical diagnosis and treatment.

In-flight medical events are not uncommon. On STS-1 through STS-89, 98% of crew members reported medical events, excluding space motion sickness (R. Williams, NASA HQ, personal communication). In total, 1867 separate events were logged (1613 men, 254 women), with 141 (7.6%) being due to injury. It is estimated that the risk on the ISS of a significant event, equivalent to one requiring an emergency room visit or hospitalization, is between 1-3 events per annum. In the Russian space program, two evacuations have been precipitated by medical conditions; in both cases, the entire crews returned. The risks increase for long duration space flight and for older crew members, particularly in view of the Vision for Space Exploration which would involve travel outside LEO. In the "stand and fight" scenario, immediate crew return is not an option, and thus the development of autonomous medical care systems will be a *requirement* of future space missions.

Given the importance of maintaining crew health, and since medical events can seriously impact astronauts and missions, the Critical Path Roadmap (CPR) ranks Trauma and Acute Medical Problems (risk #43) as one of the four Type I (most severe) risks. Research on the SMS Team aligns itself most closely, albeit not exclusively, with this risk. In the development of Counter Measures (CMs) to address risk #43, and other significant biomedical risks, the SMS Team has laid out a strategic plan for the development of advanced, integrated and autonomous systems for astronaut health assessment, maintenance and medical care. This plan has initiated, and continues to foster, collaboration with NASA flight surgeons and other medical operations personnel and biomedical researchers. In September of 2004, a Retreat was organized, together with the Technology Development Team, in Houston in which representatives from JSC and other NASA Centers, managers from the NSBRI, and several flight surgeons were in attendance. At this retreat, it was decided that a major goal of the SMS and TD Teams would be to assist NASA in the development of autonomous medical care systems that would be tested and

validated on the ISS, and thus would be qualified for use on future space missions outside of LEO.

C. Topics to Address

The SMS Team recognizes that to achieve its objectives (Section IIA), and to address the Health Concerns and Hazards of space travel (Section IIB), it must (a) utilize a team approach within the context of the NSBRI CM-driven mission, (b) coordinate and collaborate with other NASA efforts in space and critical care medicine, (c) emphasize research that leads to testing and monitoring of physiological functions and CM effectiveness in healthy astronauts, and (d) develop a long range plan to work with NASA in the development of an autonomous health care research and development program that supports the Vision for Space Exploration.

The NSBRI assigned the SMS Team a mandate to develop innovative, possibly revolutionary, techniques for medical monitoring, diagnosis and treatment. To achieve these goals, several infrastructure needs have been identified. These include:

- New types of biometric sensors
- Novel medical and surgical techniques
- Robotic medical assistance systems
- Advanced drug synthesis and delivery systems
- Smart algorithms for medical data systems
- Automated decision support for training and care
- Systems engineered platforms for sensor, algorithm and effector integration

D. Team Interrelationships

As stated in section IIA, the SMS Team objectives are broad and ambitious. However, the research program needs to be focused in order to ensure clear scientific progress towards CM development. To this end, specific synergistic relationships either exist or are being developed among team projects, as well as between SMS Team projects and other NSBRI and NASA projects. These relationships are summarized in Fig. 1.

Although the SMS Team has a talented and capable list of performers, it is still one of the smallest teams in the NSBRI. Furthermore, in order to address the full gamut of potential clinical problems that are likely to arise in a long-duration space mission, a complete autonomous medical care system would require an expansion of our existing capabilities. Although some examples of the specific topics that would be of value to the team are listed above, the funded-project selection process followed by the NSBRI makes it difficult to recruit researchers and topics that would be of great value to the Team. It is hoped that a mechanism will be found that will permit Team Leaders to have a larger impact on Team development and project selection.

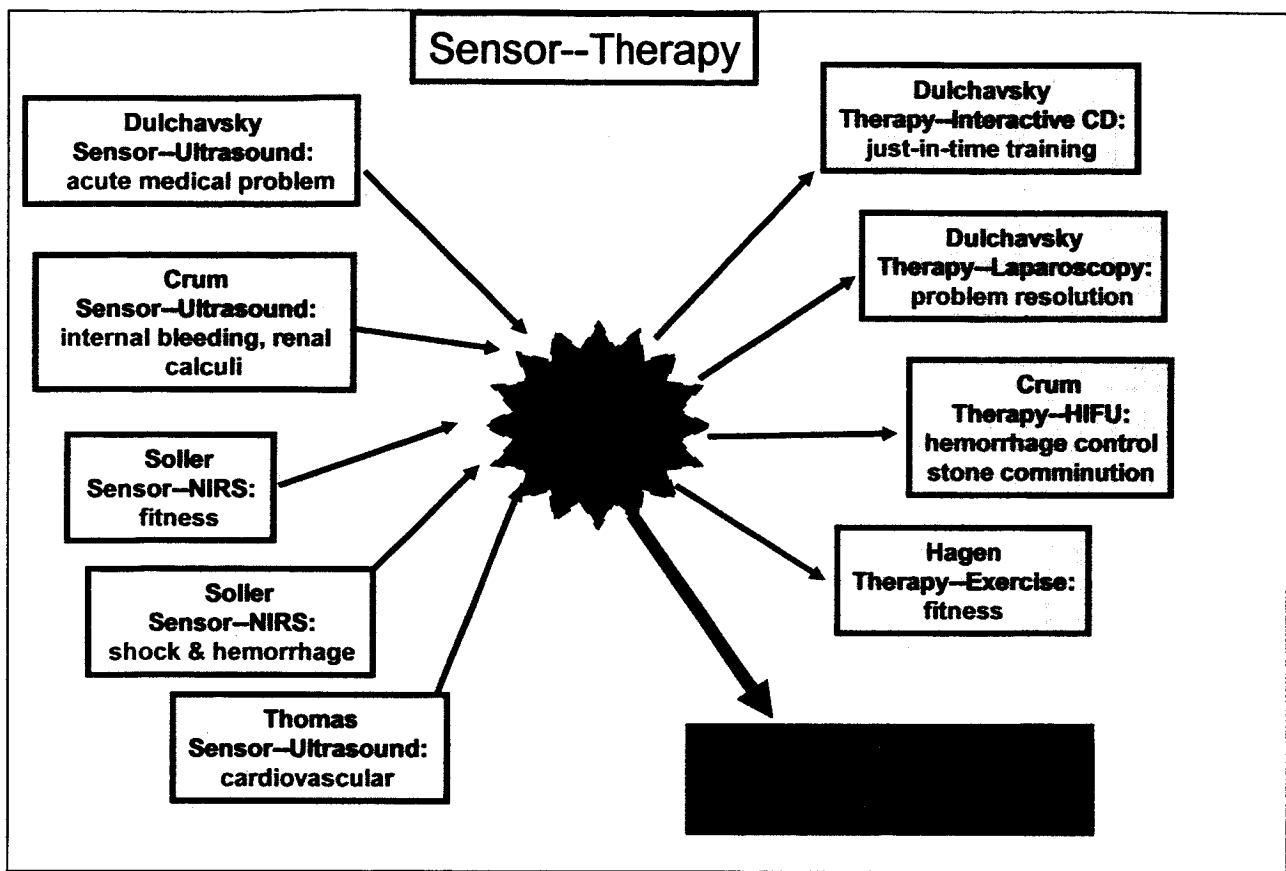
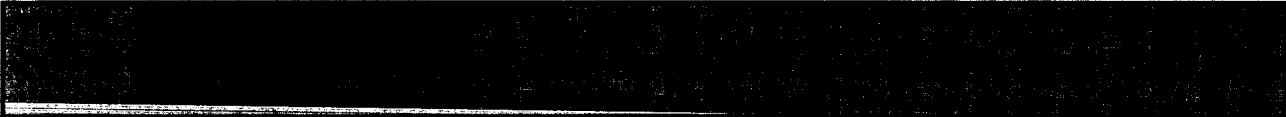


Fig. 1. Relationships between the individual projects within the Smart Medical Systems Team. The general paradigm is to use a sensor to diagnose a particular medical condition, and a therapy device to apply a countermeasure to the condition determined by the sensor. By processing the sensors through an information hub, and constructing smart algorithms for the application of therapy, this concept builds the initial infrastructure of an Autonomous Medical System—a major goal of this team.

III. TEAM ACCOMPLISHMENTS

Although SMS Team is still relatively new, considerable progress has been made. From a general perspective, the SMS Team has developed a strategic plan and coordinated research team efforts that (a) successfully interfaces with other programs within the NSBRI and NASA medical operations, and (b) has high potential impact for NASA through CM development to reduce the risks associated with long duration space travel. While funding interruptions have delayed and disrupted steady progress, effective synergies have been formed and progress has been made on each of the projects. Principal Investigator retreats have been held in April 2001, January 2003, and September, 2004. Regular telecons, multiple site visits among investigators, and education and evaluation of previous and current medical CMs have occurred. An important addition to the Team has been an Associate Team Leader, and Dr. Babs Soller has proved invaluable in building Team interactions and defining future directions. As the individual PI projects have matured, there has been movement toward higher CRLs. Indeed, Dr. Dulchavsky's project has been active on ISS Increments 8 and 9, and will continue on Increment 10. Dr. Soller's research has been extended from a measure of physiological function directed at trauma to one that permits a real-time evaluation of the effectiveness of exercise. This expansion in application has permitted it to be integrated into Dr. Hagen's exercise program underway at JSC. More specifically, the main findings and accomplishments during the past year for each project are summarized below.



Executive Summary

The principal objective of this NSBRI Smart Medical Systems Team project is to develop an image-guided ultrasound therapy system for mission critical care. In long-term space flight missions, a number of medical situations could develop that if not adequately addressed would result in mission failure. For example, although gravity is significantly reduced in space, inertia is not, and the collision of an astronaut with a heavy object could result in blunt internal trauma and its often associated internal bleeding. In addition, as recent experiences in Antarctica demonstrate, medical conditions that require some form of surgery may well appear without warning, even when extensive pre-screening is undertaken. We are developing a smart medical device that will provide a versatile capability to treat a variety of these mission-critical medical conditions. We have demonstrated that a device that produces High Intensity Focused Ultrasound (HIFU) can be combined with a device that provides ultrasound imaging to produce a duplex system that can both image a particular condition of interest and provide therapy to relieve the condition. "Image-Guided Therapy" provides enormous potential for the treatment of a variety of medical conditions. In addition, we have demonstrated that the components of such a smart medical system can reasonably be expected to be lightweight and portable.

Specific Aims

1. To expand the capability of our existing smart medical device for transcutaneous acoustic hemostasis to include a fully integrated ultrasound detection, targeting, and therapy system (CRL 5-6).

Sub aim 1a. Develop a probe applicator containing an embedded image probe together with an annular array capable of delivering HIFU at therapy levels.

Sub aim 1b. Integrate the applicator with a human interface and power unit.

Sub aim 1c. Demonstrate the capability of the integrated system to produce temperatures in excess of 75 °C at specific sites in ex vivo and phantom tissues.

Sub aim 1d. Demonstrate the capability of the integrated system to produce image-guided, transcutaneous acoustic hemostasis in animal studies.

2. To develop the capability of the integrated image-guided therapy system in SA 1 to target and ablate specific tissue volumes such as benign and malignant tumors (CRL 4-5).

Sub aim 2a. Develop a real-time guidance control and targeting system that permits HIFU lesions to be produced at specific sites in the ultrasound image of a particular region of tissue.

Sub aim 2b. Develop a lesion-imaging technique to permit real-time monitoring of the HIFU lesion produced by the therapy array.

Sub aim 2c. Demonstrate the capability of the integrated system to ablate specific sites of interest in ex vivo and phantom tissues.

Sub aim 2d. Develop a therapy protocol based upon a validated numerical model, and use this protocol to demonstrate the capability of the integrated system to ablate specific sites of interest in vivo.

3. To develop the capability of the integrated image-guided therapy system in SA 1 to target and comminute renal calculi (CRL 3-4).

Sub aim 3a. Determine through both modeling and in vitro experimentation the acoustic protocols required to produce cavitation levels sufficient to comminute artificial kidney stones without significant elevation in local tissue temperature.

Sub aim 3b. Demonstrate that the required acoustic pressure amplitudes and intensities can be produced by the therapy array of SA 1, or a related transducer system.

Sub aim 3c. Determine the minimal sizes of renal calculi that can be detected and targeted with the ultrasound imaging system of SA 1.

Sub aim 3d. Demonstrate the capability of the integrated system to target and comminute artificial stones in an in vitro model.

Results

This project involves several individuals: 5 Ph. D. Scientists, 2 engineers, 6 students (2 graduate; 4 undergraduate) and 2 visiting scientists (from Moscow State University) and is leveraged against a multi-million effort supported by the Department of Defense. Accordingly, some brief results are given below:

- The components in our image-guided HIFU system now weigh less than 7 kg, down from more than 30 kg in year 1, and 40 kg at the start of the project. They are packaged in a single chassis (a suitcase) and could operate with the Philips HDI-5000 ultrasound imager on the ISS or whatever imager is chosen for long duration missions; e.g., the “hand-held” SonoSite device or the laptop Terason device.

- We have established a protocol to sweep short-duration, high-amplitude pulses produced by the dynamic focusing transducer to treat large areas rapidly without complications from cavitation. These findings expand our trauma care capabilities, improve therapy efficiency, and minimize the power draw required by the device. When 2 frequencies are mixed, the enhancement is even greater, with hemostasis times reduced by at least 25%. Lastly, since the heated region is larger when two frequencies are used, larger vessels can be treated than were previously possible.

- We can use the same dual frequency system to treat kidney stones. Renal Stone Formation is Risk 12 on the Bioastronautics Critical Path Roadmap and the device we are currently building may be a method to comminute renal calculi that are known to form in microgravity.

Unique Claims of the Study

A multi-purpose, image-guided HIFU system for treating a number of clinical conditions that are likely to arise in a long-duration space mission is rapidly evolving toward becoming a major component of an Autonomous Medical Care System. The Intellectual Property that has been developed under this total program has attracted a number of investors. As of this date, three start-up companies have been formed in the Seattle area, and are making steady progress toward developing commercial devices. It is hoped that within a few years, Commercial Off-The-Shelf (COTS) devices will be available to NASA for use in future space missions.

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V. A. Khokhlova M. R. Bailey, J. A. Reed, B. W. Cunitz, P. J. Kaczowski, and L. A. Crum, “The relative role of nonlinear ultrasound propagation and cavitation in acceleration of HIFU therapy,” *J. Acoust. Soc. Am.*, (in preparation).

N. R. Owen, M. R. Bailey, J. Hossack, L. A. Crum, “Detection of Acoustic Signals for Synchronizing Commercial Ultrasound Imagers with a High Intensity Focused Ultrasound Therapy System,” *IEEE-UFFC Transactions*, (in preparation).

M. R. Bailey, J.A. Reed, A. D. Maxwell, W. Kreider, V. A. Khokhlova, V. Zderic, S.Vaezy, and L. A. Crum, “Dual frequency mixing to accelerate acoustic hemostasis,” *4th International Symposium on Therapeutic Ultrasound*, (Kyoto, Japan) (accepted).

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M. R. Bailey, N. R. Owen, and L.A. Crum, "Smart ultrasound therapy for mission critical care," NSBRI Investigators workshop (Houston, TX, Jan 2004) demonstration and poster presentation.

V. A. Khokhlova, M. R. Bailey, J. A. Reed, and P. J. Kaczkowski, "The relative effects of cavitation and nonlinear ultrasound propagation on HIFU lesion dynamics in a tissue phantom," *J. Acoust. Soc. Amer.* 115 (2004).

PRINCIPAL INVESTIGATOR:	Scott A. Dulchavsky MD, Ph.D.
PROJECT TITLE:	Minimally Invasive Diagnosis and Therapy of Microgravity Medical Contingencies

Executive Summary

The diagnosis and management of acute health problems in space is problematic due to limited training of the Crew Medical Officer (CMO), human and environmental factors, and a lack of reference of the changes in anatomy, disease presentation, and therapy in micro-gravity. There is no planned radiological capability aboard the ISS further complicating medical diagnosis in space. Recent terrestrial investigations suggest expanded clinical applications of ultrasound and laparoscopy with miniature instrumentation which have applicability for aerospace medicine. This proposal will initially determine the diagnostic utility of ultrasound and/or micro-laparoscopy in select health contingencies with high potential mission impact. These diagnostic modalities will then be used to facilitate minimally invasive, definitive surgical therapy of selected contingencies in animal models in ground based and simulated micro-gravity scenarios. Optimal just-in-time training regimens and computer based refresher modules for non-physician CMO's to accomplish these tasks will be developed. The unique constraints imposed by training and equipment limitations and the space environment require the development of novel diagnostic and therapeutic strategies for crew member health problems including the expansion of ultrasound and mini-laparoscopy. Thoracic ultrasound, initially investigated by NASA as an

alternative diagnostic modality for pneumothorax, has proven accuracy in terrestrial and micro-gravity applications and will have widespread impact in acute care on Earth in the future. Although some of the techniques investigated in this project are appropriate only for a micro-gravity environment, the majority of the diagnostic and therapeutic algorithms are readily transferable to terrestrial medicine including rural and military applications. The expanded use of the diagnostic and training modalities under investigation, if verified, would provide a significant, clinically relevant advance in space medical capabilities with profound Earth-based ramifications.

Specific Aims

- What is the sensitivity and specificity of ultrasound or micro-invasive laparoscopy performed by experts versus just-in-time trained CMO's in the diagnosis of serious health contingencies which may occur during space exploration?
- What are the alterations in disease presentation, diagnosis, and human factor requirements for use of ultrasound/ mini-laparoscopy in micro-gravity?
- What are the training and support requirements for physician and non-physician CMO's for optimal on site and tele-medical diagnosis of these clinical conditions by ultrasound or video mini-laparoscopy?

Results

We have developed a multi-media, interactive program (OPE: Onboard Proficiency Enhancement) which allows just-in-time training paradigms to be used to rapidly train non-physician personnel in complex medical tasks. This training program has been used in the following experimental programs. (1) Microgravity investigations on the KC-135--Non-medical personnel were able to successfully complete ultrasound and micro-laparoscopic investigations on animal models of space relevant disease. (2) Ground based studies--We have preliminary data which demonstrates that ultrasound can be performed by non-radiologists to diagnose dental and sinus pathology and musculoskeletal investigations. (3) Spaceflight--The OPE program has been used by the ISS crews of Increment 8-11 to learn ultrasound.

Unique Claims of Study

The modular development of an Onboard Proficiency Enhancement (OPE) program has allowed complex science to be conducted on the ISS with minimal crew training requirements.

Publications and Presentations

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Whitsett P, Dulchavsky SA, Hamilton D, Sargsyan A, Melton S. FAST at Mach 20: Clinical Ultrasound on the International Space Station.. *J of Trauma*, Accepted, in press.

Dulchavsky, SA. Diagnostic laparoscopy and ultrasound. Oral Keynote Lectures/Panels ISS Science Update, Houston, TX, 9/12/2003.

PRINCIPAL INVESTIGATOR:	Babs R. Soller, Ph.D.
PROJECT TITLE:	Noninvasive Measurement of Blood and Tissue Chemistry

Executive Summary

The long term goal of this project is to create a noninvasive and lightweight system for measuring blood and tissue chemistry through the skin. The technology is based on Near Infrared Spectroscopy (NIRS) and is targeted to work on subjects of any ethnic origin. The system developed during this project will measure three metabolic parameters: muscle pH, muscle PO₂ (partial pressure of oxygen) and blood hematocrit. Such a system is planned to be part of smart medical systems used to (1) identify and treat traumatic injury, (2) assess fitness levels in on earth and in space and (3) to assist in the development of exercise countermeasures to diminish loss of muscle mass, strength and endurance during long duration space flight.

Specific Aims

Original

1. Develop methodology to statistically describe NIRS spectral variations due to ethnicity, fat content and light scattering.
2. Develop and validate noninvasive methods for determining tissue pH and oxygenation from the NIRS spectra of human subjects.
3. Establish algorithms for the use of tissue pH, oxygenation and hematocrit measurements to indicate shock and hypovolemia and to guide resuscitative treatment.
4. Specify requirements for miniature, lightweight optical device.

Added

5. Design of the optical system for measurement on the leg.

Results

We have shown that near infrared spectroscopy (NIRS) can be used to simultaneously measure muscle pH, muscle PO₂ and blood hematocrit noninvasively through the palm of the hand. A small, lightweight and portable monitor with excellent stability was built for this purpose. We have demonstrated that muscle pH and PO₂ are very sensitive to small changes in peripheral perfusion induced by mild shock and that these parameters can be used to indicate severity of hemorrhagic shock and may provide a metabolic guide to resuscitation. We suggest that this tool could be used as part of a smart medical system for helping to diagnose internal bleeding and direct treatment to rescue injured astronauts.

The same monitor, with slight modifications, and a different fiber optic cable can be used to monitor metabolism of specific muscles during exercise. Such a device could be used quantify fitness on the ground, changes in space, and the effectiveness of exercise countermeasures. This year we began the design of the modified monitor and fiber optic cable for measurement of leg muscle during cycling exercise. This work is aimed at developing a system to be used by NASA and NSBRI exercise physiologists.

The unique, portable spectroscopic monitor could be used on a day-to-day basis to assess astronaut fitness and also be available for emergency medical care. Since astronauts will be familiar with its operation for exercise, use in an emergency situation would require minimal training, especially when combined with software to assist in diagnosis and treatment of trauma.

Unique Claims of Study

There are no commercially available systems which can noninvasively measure metabolic parameters that can be used to diagnose and treat shock and internal bleeding. Similarly, there are no noninvasive systems which can continuously monitor muscle anaerobic metabolism for use in assessing exercise performance, training and muscle atrophy. This project has demonstrated the feasibility of such a device. Not only have we demonstrated the technology to noninvasively measure muscle pH, PO₂ and blood hematocrit, but we have developed methods which will help ensure that the measurements are accurate for people of all ethnic origins and on parts of the anatomy that are covered with thicker fat layers.

Publications, Patents, and Presentations

Landry MR, Soller BR, Previte GJ: Alignment Target for Precise Probe Placement. Provisional patent application.

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Phillipps P, Phillipps M, Soller BR: Stabilized Filament Light Source. Provisional patent application.

Soller BR, Strangman G, Sutton JP: Applications for Near Infrared Spectroscopy and Imaging in Assessing Astronaut Health Status, J Biomed Optics, *submitted*

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Soller BR, Idwasi PI, Heard SO: Noninvasive Measurement of Tissue Oxygenation Parameters Using Near Infrared Spectroscopy. USARIEM, US Army, Natick, MA, February 2003.

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Soller BR, Heard SO: Noninvasive Measurement of Blood and Tissue Chemistry
NSBRI/NASA Investigator's Meeting, Houston, TX, January 2004.

Soller BR, Phillipps P, Phillipps M, Soyemi O, Heard SO: Near Infrared Spectroscopy to Assess Metabolic Parameters to Guide Resuscitation from Shock. Smart Medical Technology Summit, Houston, TX, April 2004.

Soller BR, Phillipps P: Portable, Noninvasive System for Use in the Triage and Treatment of Shock: System Design and Characterization. Military Health Forum, San Juan, PR, April 2004.

Soller BR, Idwasi PO, Balaguer J, Levin S, Simsir SA, Vander Salm TJ, Collette H, Heard SO. Noninvasive, NIRS-Measured Muscle pH and PO₂ Indicate Tissue Perfusion for

Cardiac Surgical Patients on Cardiopulmonary Bypass. Critical Care Medicine. 31, 2324-2331 (2003).

Soller BR: Noninvasive Measurement of Tissue Oxygenation. Application No.: 10/269,826. This patent was allowed and will be issued in May, 2004.

Soller BR and Idwasi IP: Correction of Spectra for Subject Diversity. Pending. Application No.: 10/086,917. Converted from provisional to utility patent.



Executive Summary

The cardiovascular system undergoes significant changes in microgravity, including an early cephalad shift of lower extremity blood volume, loss of plasma volume over 24 to 48 hours, and long-term reduction in ventricular chamber volume and mass. In the weightless environment, these alterations are generally well tolerated, but upon return to earth, astronauts often suffer from serious orthostatic intolerance and reduced exercise capacity, changes that may limit the long-term presence of man in space. It is essential that the mechanisms for these alterations be understood so that reliable countermeasures can be tested and implemented. Hypovolemia, cardiac atrophy, and autonomic dysfunction have each been hypothesized to contribute to this post-flight debility, but their relative importance is unclear. Furthermore, it is unknown whether actual abnormalities within the myocardium develop with long-term space flight. Therefore, reliable portable noninvasive methods will be needed in order to detect and quantify these changes. Alone among imaging modalities such as radiography, magnetic resonance imaging and computerized tomography, echocardiography has the unique ability to characterize cardiovascular anatomy and physiology in ground-based models, pre- and post-flight, and most importantly during flight. Our central hypothesis is that microgravity affects cardiovascular function not only through changes in chamber volume and mass but also through changes in myocardial properties. A definitive test of this hypothesis is at least several years away when dedicated life science missions are possible aboard the ISS.

Specific aims

- Assessment of the effect of chronic volume and pressure unloading on ventricular myocardial properties
- Validation of non-invasive Doppler echocardiographic indices for the assessment of left ventricular contractility and relaxation including color M-mode Doppler derived diastolic intraventricular pressure gradients (IVPG) and tissue Doppler derived myocardial systolic and diastolic strain rates
- Validation of Doppler derived exercise cardiac output and contractile reserve and their potential utility for the *early detection* of myocardial dysfunction during prolonged space flight
- Development and distribution of stand-alone software and algorithms for implementing the quantitative analysis of Doppler echocardiographic data, as described above, so they may be applied to ultrasound data obtained from remote sources
- Establishment of an Echocardiographic Core Facility for the NSBRI and NASA community, capable of applying standard and novel analysis techniques to data obtained from

selected ground-based experimental models, pre- and post-flight examinations, and eventually from in-flight acquisitions.

If successfully implemented, these Aims will allow the cardiovascular sequelae of space flight to be studied much more rigorously, while providing consistent, objective echocardiographic interpretation to the entire NASA community.

Results

Clinical Significance of 3D Ultrasound Capabilities: To date we have performed over 2500 patient examinations with real-time 3D echocardiography, including exercise and intraoperative (epicardial) examinations, with quantitative validation in aneurysmal ventricles, aortic regurgitation, hypertrophic cardiomyopathy, mitral regurgitation, and dilated cardiomyopathy. We validated 3D color Doppler stroke volume as well as 3D reconstruction using a device identical to the ultrasound system on the ISS. Building on our experience with the Volumetrics RT3DUS, we have begun to use a much improved acquisition device (Sonos 7500) to obtain over 100 RT3D examinations in a wide variety of cardiac pathologies, including 28 acquisitions obtained epicardially in the operating room. Already, six abstract presentations and a journal article have resulted from this work including volume assessment validation, valvular assessment for surgical planning compared to standard 2D imaging, and surgical outcome results of myectomy in patients with hypertrophic cardiomyopathy.

Registration of Resting Cardiac Ultrasound and SPECT: We have also performed MI-based registration of gated Sestamibi SPECT and RT3D ultrasound images in a pilot study involving nine subjects. Recently, the image quality of 3D ultrasound has improved significantly. We will further explore cross registration capabilities in Specific Aim #2.

Impact of Volume/Pressure unloading on LV properties: As a model for LV mass regression in space, we have studied the dramatic LV regression that occurs when patients are relieved of their pressure or volume overload following aortic valve replacement for aortic stenosis or regurgitation. We had thus far enrolled sixteen patients with aortic stenosis and eight with aortic insufficiency, all of whom have undergone initial three-dimensional echocardiography but only nine have undergone follow-up. After aortic valve replacement, the peak and mean pressure gradient across the aortic valve significantly decreased. During follow-up, there were no significant changes in left ventricular end-systolic volume and ejection fraction, both $p > 0.05$ vs. baseline data. However, the left ventricular end-diastolic volume, left ventricular myocardial volume and mass significantly decreased at follow-up time ($p < 0.01$). Furthermore, we have looked at sixteen patients with hypertrophic cardiomyopathy undergoing septal myomectomy and have shown a decrease at six months, far in excess of the amount of septal tissue resected, indicating the very significant degree of secondary hypertrophy that occurs in hypertrophic cardiomyopathy due to the left ventricular outflow tract obstruction.

Noninvasive Echo Assessment of LV Function: We have continued rapid development of several new echocardiographic indices for quantifying systolic and diastolic function. We have demonstrated that myocardial systolic strain-rate is a noninvasive surrogate for the end-systolic elastance, and have demonstrated regional variance in strain to be a powerful measure of success in biventricular pacing. We have assessed the preload and inotropic dependency of tissue

velocity in dogs and have shown that contrary to the popular notion, systolic tissue velocities in the normal heart are not independent of preload, this state only being observed in pathologic hearts or in hearts with delayed relaxation. We confirmed similar findings in humans undergoing a microgravity mimicking bedrest in studies that we have collaborated on with Dr. Ben Levine in Dallas, Texas.

We have also completed much of our preliminary validation work for measuring the intraventricular pressure gradient (IVPG) from application of the Euler equation to color M-mode Doppler echocardiographic data of transmitral flow. We have used this to quantify the changes in diastolic suction following septal ablation in hypertrophic obstructive cardiomyopathy, a presentation that won the Young Investigator's Competition for the American College of Cardiology in 2002. We have also shown that the color M-mode propagation velocity is more independent of preload than tissue Doppler velocity, making it an attractive index to assess cardiovascular countermeasures in space.

Exercise Assessment for Early Detection of Myocardial Dysfunction during Prolonged Spaceflight: We have conducted a number of clinical studies demonstrating the efficacy of exercise echocardiography in detecting subclinical disease. In a recent study, we studied 31 patients with heart failure and 15 normals before and after submaximal metabolic stress testing, applying the Euler equation to their color M-mode Doppler data to calculate IVPG. [Rovner et al, *J Am Soc Echocardiogr* 2002] Although the resting IVPG was only weakly associated with the maximal exercise capacity ($\text{VO}_2 \text{ max}$), the increment in IVPG was the best predictor of exercise capacity ($r=0.80$, $p<0.001$). We have also published a validation demonstrating the use of a simplified index of cardiac power as a way to monitor cardiac reserve, a very simple approach that may have important application in space.

Assessment of Myocardial Mechanics using 2D Echocardiographic Strain measurement: In the past 3 years, our group and others have worked to develop echocardiographic measurements of LV strain. [Greenberg et al., *Circulation* 2002] Currently, this is done by differentiating in space and integrating in time a tissue velocity map, an approach that has proven valuable in studying patients with coronary disease, cardiomyopathies, and diastolic dysfunction. However, this Doppler-derived strain is highly sensitive to signal noise and the angle of ultrasound interrogation and only provides a single strain component. Extraction of myocardial mechanics by "speckle tracking" of 2D echos was proposed almost 20 years ago, but accurate quantification of regional 2D strain has become feasible only recently with the advent of high-resolution, high-frame rate grey-scale images. A comprehensive assessment of cardiac motion and deformation can be achieved providing myocardial tissue velocities, strain rates and strains without any angle dependency. Our initial experimentation with this algorithm has been very encouraging regarding its utility and accuracy. Regional velocity and strain measurements have been compared in 80 individual segments from 5 subjects using standard tissue Doppler measurements and 2D speckle tracking. A strong correlation ($r=0.79$) between these approaches has been observed, the discrepancy likely reflecting that the principle (maximum) strain vector can be obtained only in 2D, while the tissue Doppler approach is limited to the component along the ultrasound beam.

Echocardiographic CORE facility for NSBRI and NASA:

We continue to be quite busy in our core analyses for the NSBRI funded bedrest studies in Boston (Richard Cohen: PI) as well as NASA funded work in Dallas (Ben Levine, PI). We are also collaborating with Dr. Lakshmi Putcha (Johnson's Space Center), to quantify hepatic flow as well as to Drs. Lorell and Schneider concerning the measurement of cardiac strain in rodent models of microgravity. Through the Smart Medical Care Team, we are also working with Dr. Lawrence Crum to couple improved ultrasound diagnostics with his high intensity focused ultrasound (HIFU) method for cauterization and tumor ablation.

Unique Claims of the Study

- Ultrasound images can be successfully acquired with the ISS ultrasound unit and relayed to ground
- 3D echocardiography lends unique insight into cardiac anatomy and function
- Wavelet packet compression can be applied to 3D ultrasound at ratios > 100:1
- 3D echos, even post-compression, can be segmented to yield cardiac cavities and surfaces
- Cross-modality 3D image registration is possible, allowing the monitoring of physiological and pathological changes in space
- Digital echo storage and retrieval accomplished at CCF, >200 studies/day, 10 GB/day
- Ultrasound data can now be transmitted wirelessly using industry standards at full diagnostic resolution.

Publications and Presentations

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Khot UN, Novaro GM, Popovic ZB, et al. Nitroprusside in critically ill patients with left ventricular dysfunction and aortic stenosis. *N Engl J Med.* 2003;348:1756-63.

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IV. TEAM PLANS

Team integration. Efforts have been underway since the initial composition of the Smart Medical Systems Team to more fully integrate the various projects within the team. However, since several of the projects were not renewed, this integration has been difficult to accomplish. After the latest round of proposal submissions and notifications of funding, a renewed attempt at integration has been made. Since SMS does not have a central goal—a specific risk or clinical problem—such as bone loss or immunology, the SMS team has been viewed more as a “stable” than a team. Nonetheless, recently we have discovered that most of the current projects either involve ultrasound, or integrate quite readily with ultrasound. Thus, we now anticipate a more integrated team and plan to work together much more closely in the past.

Team additions. The team as presently constituted is quite small, with essentially four investigators. We have made some effort to integrate the Space Medicine projects into SMS, but this may not be wise from a programmatic aspect, and is probably more of a management decision at the NSBRI Directorate level, rather than at an individual project or team level. There is a clear need, however, for additional biometric sensor projects, novel surgical techniques, robot-assisted systems, advanced drug synthesis and delivery systems, smart algorithms for medical data systems, and systems-engineered platforms for sensor, algorithm, and effector integration.

Autonomous medical care. The evolution of medical care in light of the vision for space exploration must eventually turn toward autonomous systems that can operate independently of space-to-earth communications, as well as of complete training of astronauts for all medical

emergencies or contingencies. Smart Medical Systems, examples of which are being developed by this team, are microcosms of a more general autonomous medical care system. We hope that NASA will appreciate the progress we have made so far in this team and permit us to assist in the development of a major program in autonomous medical care, not just for the NSBRI, but for NASA as well.

ANNUAL TEAM REPORT—TECHNOLOGY DEVELOPMENT

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Project: Improved Bubble Detection for EVA

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**Project: Ground-Based Measurement of Bone Loss in Astronauts Using the AMPDXA
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Project: Combined Ion and Neutron Spectrometer (CINS) for Space Application

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Project: MicroDosimeter iNstrument (MIDN) Suitable for Spaceflight

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Project: Miniature Time-of-Flight Mass Spectrometer

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Project: A Scanning Confocal Acoustic Diagnostic System for Noninvasively Assessing Bone Quality

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Project: Handheld Body-Fluid Analysis System for Astronaut Health Monitoring

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I. ABSTRACT

The technology development team projects focus on monitoring, preventing or diagnosing biomedical problems in space. Two of the projects are radiation monitors. One, the **neutron spectrometer**, provides information on the spectrum of radiation entering the instrument. The other, the **microdosimeter**, measures the amount of energy deposited by radiation in cell-sized detectors. These detectors are complementary, since information on both radiation spectra and absorbed radiation are important for spaceflight. Two projects are concerned with space-related bone loss, which must be slowed or stopped to allow for long-duration spaceflights. The **AMPDXA** is a small dual-x-ray absorptiometry device that could be used to measure bone loss in astronauts. The **acoustic diagnostic system** detects changes in bone mass and bone quality using ultrasound, and is well suited for use in space. Three projects provide diagnostic information. The **small MRI**, while designed for research on small animals, could also be adapted to provide medical images on limbs. The **bubble detector** provides a new way to detect and size the bubbles of decompression sickness in both blood and tissue (decompression sickness is a risk during spacewalks). The **handheld body-fluid analysis system**, which employs microelectromechanical system (MEMS) technology, could provide both important clinical diagnostic information and data on immunological changes in space. One project is a versatile technology that could be used for both monitoring and diagnosis. This technology, the **time-of-flight mass spectrometer**, can measure compounds over a wide mass range and could be applied to measuring various compounds in air, water, blood or urine.

All projects made good progress during the reporting period. Most projects have links with groups at NASA that are interested in the technology. Several projects could meet an immediate need at NASA (radiation monitoring and environmental monitoring), and others could meet important needs once further development is completed (bone loss monitoring, bubble detection, clinical diagnosis). None, however, is moving toward flight utilization. The most significant challenge facing the team is defining a clear pathway to bring a mature technology to flight.

II. INTRODUCTION

To realize the goals within the Vision for Space Exploration, the most important service the biomedical program can provide is prevention. Although the provision of medical care is important, the most efficient way to accomplish the exploration mission is to keep significant medical and physiological events at the lowest level possible. Accomplishing this goal requires effective monitoring. For example, treating a kidney stone in space is possible, but requires a significant overhead in equipment, training and supplies. If, however, urinary composition could be monitored during a spaceflight, then interventions could be used to keep the risk of stone formation extremely low. Similarly, if bone loss, radiation levels, air contaminants, immune function, and the propensity to get decompression sickness could be monitored, this would give the medical team the chance to intervene before significant problems develop. Many of the technology team's projects are designed to provide effective monitoring.

Accurate diagnosis is also important in space. For example, a high white blood cell count often indicates infection, but currently no method exists to measure the white blood cell count in space. The ability to measure a white blood cell count would be helpful to crew medical officers and flight surgeons to help decide whether a particular set of symptoms (e.g. significant abdominal pain) might be associated with infection. Improved imaging technologies also allow for more accurate diagnosis. The technology develop team has projects that are focused on diagnosis. Table 1 summarizes the diagnostic and monitoring functions that the current projects have.

The team's organization is focused on providing each team member the knowledge and contacts they need to move their technology forward within the NASA system. Each team member is encouraged to find partners at NASA centers, the NSBRI, or with other NASA-supported researchers, to advance their technology. Table 2 lists some of the collaborative arrangements.

<i>Project</i>	<i>Monitoring Function</i>	<i>Diagnostic Function</i>	<i>Notes</i>
Space MRI system	Possible to measure muscle changes	Medical imaging	Could be used to image limbs
Bubble Detector	Precursor bubbles in tissue may correlate with decompression sickness risk	Assess presence and severity of decompression sickness	Provides bubble size information, which is a new capability
AMPDXA	Follow bone loss in astronauts	Can diagnose osteoporosis	Current system is ground-based, but could be adapted to flight

<i>Project</i>	<i>Monitoring Function</i>	<i>Diagnostic Function</i>	<i>Notes</i>
Ion and Neutron Spectrometer	Radiation spectrum in spacecraft, including high energy neutrons		The ability to measure high energy neutrons is an advance this device offers
Microdosimeter	Absorbed radiation within spacecraft		Rugged, portable, low power, lightweight
Time of Flight Mass Spectrometer	Air quality, water quality, bone loss markers, markers of oxidative stress	Could measure a variety of diagnostically important molecules in blood and urine	High technology readiness
Confocal Acoustic Diagnostic System	Follow bone loss in astronauts	Can diagnose osteoporosis	Portable, ultrasound-based
Handheld Body-Fluid Analysis System	Follow white blood cell count	Diagnose infection	Currently focused on measuring white blood cells. Other analyses possible in future. Uses MEMS technology — very small

Table 1: List of potential monitoring and diagnostic applications for the NSBRI Technology Development Team projects.

<i>Project</i>	<i>Collaborative Arrangement</i>
Space MRI system	Radiology Department, Johns Hopkins
Bubble Detector	NASA-JSC, Drs. Gernhardt and Powell
AMPDXA	Commercial partner
Ion and Neutron Spectrometer	NASA-Marshall, Los Alamos and others
Microdosimeter	NASA-JSC, Dr. Cucinotta
Time of Flight Mass Spectrometer	NSBRI Bone Team, NSBRI Behavioral Health and Performance Team, Homeland Security
Confocal Acoustic Diagnostic System	NASA-JSC, Bed rest team
Handheld Body-Fluid Analysis System	NASA URETI Center at UCLA

Table 2: Collaborative arrangements in place for the various projects both within NASA and outside of NASA.

III. TEAM ACCOMPLISHMENTS

OCTOBER 1, 2003 – SEPTEMBER 30, 2004.

Key findings and milestones (copies of presentations from the individual projects at the annual team review are included as an Appendix)

Bankman (Space MRI):

Development of superconducting magnet and gradient amplifier was begun. Initial images were taken from the existing system. Image processing software for the space application has been developed.

Buckey (Bubble detector):

Histograms of bubble sizes have been created in-vivo, and data suggest these may change with interventions. Detailed in-vitro calibration is ongoing, which includes an assessment of factors that can influence bubble detection and sizing. A digital implementation of the device has been completed.

Charles (AMPDXA):

Verified detector-source geometry of the AMPDXA-GCS using three fixed projection angles over a 30° arc (0°, +/-15°) rather than the 90° arc for the original AMPDXA. AMPDXA measurements compared to actual dimension on artificial bone cylinders, with excellent results. Repeatability measurements (5 sequential measurements) performed which show good repeatability

Maurer (Neutron Spectrometer):

During a successful balloon flight in October 2003 determined the downward neutron energy spectrum at 85,000 feet (20 grams per square centimeter) an atmospheric depth equivalent to the Martian surface. This neutron energy spectrum will assist in predicting the radiation dose equivalent due to neutrons on Mars. The Technology Readiness Level (TRL) of the instrument advanced to 6 through the balloon flight activity. Accelerator shielding experiments show that neutron production by high energy protons on thick targets simulating large spacecraft is greatest for aluminum and reduced for carbon based materials, polyethylene and combinations of carbon and polyethylene. This result provides guidance for constructing shelters with the best radiation protection.

Pisacane (Microdosimeter):

Developed prototype instrument to carry out test and calibration activities. A preliminary version will fly in a student-developed spacecraft for the Department of Defense Space Test Program. Approximately 13 microdosimeter sensors are under development at the Centre for Medical Radiation Physics, University of Wollongong. They will be evaluated based on I-V characteristics, C-V characteristics, and response to alpha source Po-201. Two low noise power supplies are being designed and produced to power the instrument. Development of the testing protocols for the instrument has been initiated. Fluence spectra as a function of LET and atomic number were calculated and will be used in the development of an analytical response model of the MIDN detector.

Potember (Time-of-flight Mass Spec):

Demonstrated that cross-linked N-telopeptides of type-I bone collagen (NTx) can be

measured using the TOF Mass Spectrometry. We are investigating the time of flight mass spectrometer as a diagnostic tool to measure human bone resorption rates, utilizing N-telopeptides in urine as the test specimen. We are currently analyzing urine samples from Dr. Kenneth P. Wright Jr. for the excretion of the melatonin metabolite, 6-sulphatoxymelatonin. Device improvements have been made.

Qin (Acoustic diagnostic system):

An experimental and clinical prototype of the scanning confocal acoustic diagnostic (SCAD) system, including hardware and software, has been developed. New micro-controller guided acoustic scanning technology has been incorporated that reduces the scan time markedly, e.g., 0.5~1 mm resolution images take only approximately 3-4 minutes. A surface topology extraction method has been designed for identifying the bone surface features. Bone surface features from both sides of the bone are determined simultaneously during confocal scanning with a mapping resolution on the order of 2 microns. The feasibility of SCAD bone quality assessment for bone quality has been evaluated in the calcanei of cadavers. Strong correlations were found between BUA and bone volume fraction (BV/TV) ($R^2 = 0.76$), and between ultrasound velocity and the bone's modulus ($R^2 = 0.53$). The correlations are significantly improved ($R^2 > 0.64$) using combined parameters of BUA and UV in linear regression with structural parameters determined from ultrasound images, e.g., structure morphological index (SMI) ($R^2 = 0.86$), and strength modulus ($R^2 = 0.64$). These results suggest that high-resolution acoustic mapping is capable of predicting calcaneal bone quantity and quality non-invasively.

Tai (Handheld Body Fluid Analysis System):

Designed and fabricated a new MEMS device based on the deterministic lateral displacement principle and achieved continuous separation of erythrocytes (red blood cells, RBCs) and leucocytes (white blood cells, WBCs). The device has a chip area of 1cm by 1cm with an effective separation area of 7mm by 1.8mm. RBCs and WBCs are separated based on size. Calibrated with polystyrene beads, the critical size for separation for the current device is about 8 μ m. Testing on human blood separation has been performed with both blood fraction with concentrated WBCs and whole blood. Solvent Ficoll-Paque Plus, which has a density close to whole blood, is used to dilute the human blood sample. A separation of 400 μ m is achieved around the outlet of the device.

Significant scientific/technology/education papers

Buckey, JC. Knaus, D.A, Alvarenga DL, Kenton MA, Magari PJ. Dual Frequency Ultrasound for Detecting and Sizing Bubbles. Acta Astronautica, (in press)

J. D. Kinnison, R. H. Maurer, D. R. Roth, P. J. McNulty and W. G. Abdel-Kader, Neutron-Induced Pion Production in Silicon-Based Circuits, IEEE Transactions on Nuclear Science **50**, 2251-2255, Dec. 2003

R.H. Maurer, J. D. Kinnison and D. R. Roth, Neutron Production from 200-500 MeV Proton Interaction with Spacecraft Materials, ICRS 10/RPS 2004, Madeira Island, Portugal, May 2004

V. L. Pisacane, J. F. Ziegler, M. E. Nelson, M. Caylor, D. Flake, L. Heyen, E. Youngborg, A. B. Rosenfeld, F. A. Cucinotta, M. Zaider, and J. F. Dicello, MIDN, A Spacecraft Dosimeter, 14th International Conference on Solid State Dosimetry, Yale University, New Haven, CT, 27 June to 2 July 2004. Accepted for publication in Radiation Protection Dosimetry.

Wroe, A., I. Cornelius, A. Rosenfeld. The role of inelastic reactions in absorbed dose distributions from therapeutic proton beams in different medium. Medical Physics , (in press).

Wroe, A., R. Schulte, V. Bashkirov, A. Rosenfeld, S. Shchemelin, B. Grosswendt Nanodosimetric cluster size distributions of therapeutic proton beams, accepted IEEE NSS MIC conference , Roma, 16-22 October , 2004.

Qin, Y-X., Mittra, E., Lin, W., Xia, Y., Rubin, C. (2003): Non-invasive assessment of bone strength and density using scanning ultrasound. ASME-BED Bioeng Conference Proceeding, 51:373-374.

Mittra, E., Rubin, C. and Qin, Y-X. (2004): Interrelationship of trabecular mechanical and microstructural properties in sheep trabecular bone. J Biomech, (in press).

Mittra, E S, Karnik, S, Rubin, C. and Qin, Y-X. (2004): Variability in trabecular tissue properties across bone with disparate quantity and quality. 50th Ann Mtg Orth Res Soc. Vol. 29:522.

Xia, Y., Lin, W., Mittra, E., Gruber, B., Demes, B., Rubin, C. and Qin, Y-X. (2003): Performance of a Confocal Acoustic Mapping in Characterization of Trabecular Bone Quality in Human Calcaneus. Ann Am Soc Bone Mine Res, J Bone Min Res, 18:SU118.

Mittra, E.S., Rubin, C.T. and Qin, Y-X. (2003): Characterization of Changes in Trabecular Bone with Age & Disease. IEEE-EMB, 29th Annual Northeast Bioeng. Conference, 35-36, First Place Award.

Xia, Y., Lin, W., Mittra, E., Reardon, C., Gruber, B., Rubin, C., and Qin, Y-X. (2003): Confocal Acoustic Scanning for characterizing human trabecular bone quantity and quality. IEEE-EMB, 29th Annual Northeast Bioeng. Conference, 26-27.

Mittra, E., Rubin, C. and Qin, Y-X. (2003): Interrelationship between bulk, microstructural, and material properties in sheep trabecular bone. Biomed Eng Society Annual Conference, Annals Biomed Eng, 31:

Xia, Y., Lin, W., and Qin, Y-X. (2003): Effect of Cortical Shell on the Measurement of Ultrasound Attenuation in Bone Quality Assessment. Biomed Eng Society Annual Conference, Annals Biomed Eng, 31:

Mittra, E., Karnik, S., Lin, W., Demes, B. and Qin, Y-X. (2003): Changes in nano-scale trabecular material properties in osteoporotic human cadaver calcaneus. Biomed Eng Society Annual Conference, Annals Biomed Eng, 31:

S. Zheng, R. Yung, Y.C. Tai and H. Kasdan, Deterministic Lateral Displacement MEMS Device for Continuous Blood Cell Separation, Eighteenth IEEE International Conference on Micro Electro Mechanical Systems (MEMS '05), Miami, USA, coming Jan. 30- Feb. 2 (2005).

New intellectual property

Qin, Y-X., Lin, W. and Rubin, C.T.: Frequency Scanning of Ultrasound Attenuation as a Diagnostic to Determine Bone Physical Properties. Patent Pending.

Qin, Y-X., Lin, W.: Phased-Array Electronic Confocal Ultrasound Scanning for Material and Tissue Quality. Disclosure #R-7682, 2003

Qin, Y-X., Xia, Y. and Lin, W.: Scanning Acoustic Topology Mapping for Determining Tissue Surface Features and Wave Transmit Thickness. Disclosure #R-7681, 2003
Product demonstrations

NASA collaborations (see Table 2)

Academic/industry/government partnerships

- The Bubble Detector project is a partnership between Dartmouth College and Creare Incorporated.
- The Handheld Body-Fluid Analysis Project is a collaboration among Caltech, UCLA and Iris Diagnostics. This project also shares similar technologies developed under the NASA URETI CMISE Center at UCLA.

Leveraging of resources

- The **bubble detector** project also receives funding from the Office of Naval Research
- For the **microdosimeter** project, the United State Naval Academy (USNA) has ownership of the HAWK system, a \$30,000.00 instrument procured by for dose measurement purposes in the USNA Nucleonics Laboratory. Several NIM (Nuclear Instrumentation Modules) electronic modules (power supplies, pulsers, amplifiers) will also be procured by the USNA Nucleonics Laboratory and dedicated to this project. Small scale test equipment is available such as power supplies, voltmeters, soldering equipment, etc in the Satellite Ground Station of the USNA.
- The **acoustic diagnostic system** was previously supported by the New York Advanced Center for Biotechnology and a new grant is pending.

- The **handheld body fluid analysis system** has some undergraduate students working on this project as senior thesis students. The funding comes from California Institute of technology. Also we are designing the devices as modular microfluidics. The funding of modular microfluidics comes from CMISE (Institute of Cell Mimetic Space Exploration, a NASA URETI Center at UCLA).

Synergies among projects

- Synergy exists between the **microdosimeter (MIDN)** project and Dr Dicello's NSBRI project to measure cancer risk in an animal model.
- The **microdosimeter** project is also complementary to another project in the NSBRI Technology Development Team, the *Combined Ion and Neutron Spectrometer for Space Applications (Spectrometer)* by Richard. M. Maurer of the Johns Hopkins University Applied Physics Laboratory. The Spectrometer measures the energy spectra of both charged and neutral particles. These observations are complementary to the MIDN measurements of energy deposition in silicon. To assess risk, given the spectra observations from the Spectrometer, it is necessary to model the energy deposition in tissue and then assess the Relative Biological Effectiveness (RBE) or radiation quality factor (Q). Given the observations of deposition of energy as measured by MIDN, it is only necessary to model the difference between the effects of the radiation in silicon and tissue. These complementary observations and simulation will provide an opportunity to determine the consistency between the two approaches. In addition, the MIDN is a low powered, low mass system that is portable and can potentially be made into a personal dosimeter worn by astronauts, although that is not a goal of this research project.

Advances toward testing in flight

- The Scanning Confocal Acoustic Diagnostic system technology has been involved in a NASA flight project, and will be used for pre- and post-flight bone quality measurement for astronauts.

Space and earth implications of the current accomplishments

- A small, low power consumption small MRI system could be useful in other field research applications.
- The bubble detector technology will also be useful to the diving community.
- A small, low power consumption DXA machine could be useful in clinical settings.
- The neutron spectrometer is helping to test shielding materials.
- A microdosimeter could be used wherever radiation exposure is a risk.
- The time-of-flight mass spectrometer is currently employed in monitoring the environment for homeland security applications.
- An acoustic diagnostic system that measures bone quality would be useful clinically.
- A handheld body-fluid analysis system would have a wide variety of clinical applications. The device could find applications in emergency rooms or ambulances thanks to its small size and fast measurement time.

IV. TEAM PLANS

The main goal for the team over the next year is to move the mature technologies into flight development. The **time-of-flight mass spectrometer** and the **neutron spectrometer** are mature technologies that could meet immediate needs at NASA. The existing air and water quality monitoring systems on the space station are currently not functioning. The time-of-flight mass spectrometer could potentially monitor air and water quality in space and offer significant advantages over the current techniques. Also, there is no good method at present on the space station to measure high-energy neutrons—and neutrons form a significant part of the radiation dose received on the station. The neutron spectrometer could meet this need. The goal for the next year is to establish the pathway to move these projects to flight. Ultimately this will help all the projects, since any project that reaches a high technology readiness level (i.e. any successful project) will face the same set of problems in moving the device to the next step.



Space-Qualifiable MRI System



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Paul A. Bottomley

Consultant; MRI instrument design

Vadappuram P. Chacko

Consultant; MRI imaging and data

Resonance Research Inc.

Billerica, MA

Piotr Starewicz

Physicist; MRI magnet design

Project status: Ongoing, year 2

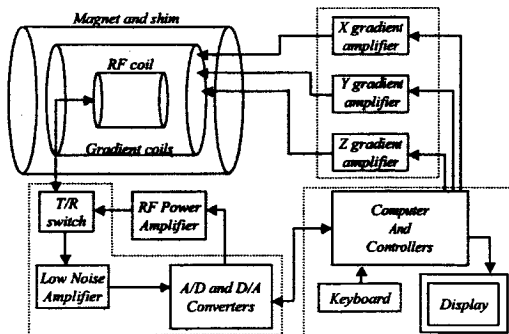
1



Introduction



1. Contribute to the study of physiological and anatomical changes in the space environment
2. Facilitate the development and evaluation of countermeasures
3. Monitor the effectiveness of countermeasures



Applications:

- Bone Loss
- Muscle Alterations and Atrophy
- Cardiovascular Alterations
- Nutrition
- Physical Fitness and Rehabilitation
- Smart Medical Systems

Resolution requirement	Weight constraint	Power constraint	Feasible bore diameter
0.2 mm	150 kg	1.2 kW	45 mm

2



Development approach



1. Configure baseline MRI system with commercial parts
 - Console, amplifiers, gradient and RF coils, magnet and shims
2. Modify components that are not space-qualifiable
 - Cooling system of magnet (fluid refill and venting)
 - RF signal amplifier (high power consumption)
 - Gradient amplifiers (high power and weight)
3. Integrate new parts and evaluate the system
 - Image quality analysis
 - Best mode for MRI image acquisition
 - Demonstration on mice
4. Develop image analysis software to measure muscle and bone loss
 - Enhancement
 - Segmentation
 - Quantification
 - Registration

3



Three-year view



Year 1 progress

Baseline MRI system



Magnet design

1 Tesla
50 Kg
1 KW



RF amplifier development

MRI system simulation

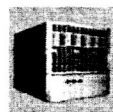
$$SNR = \frac{\left(B_0 \rho_p \left(\rho e^{pT_1/T_1} \right)^{pT_2/T_2} \right)}{\left(B_0 \rho_r \left(\rho e^{pT_1/T_1} \right)^{pT_2/T_2} \right)}$$

Image quality assessment software (Development module)



Year 2 progress

Superconducting magnet development started



Gradient amplifier development started

Initial images

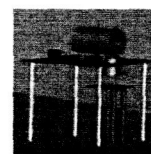


Image processing software for space application (Operational module)

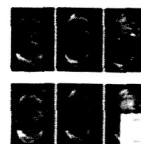


Year 3 plan

System integration



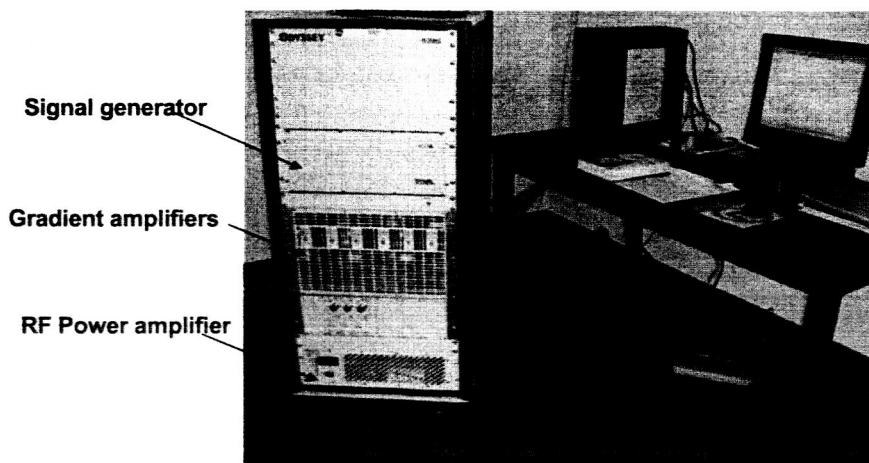
Testing and evaluation on mice



4



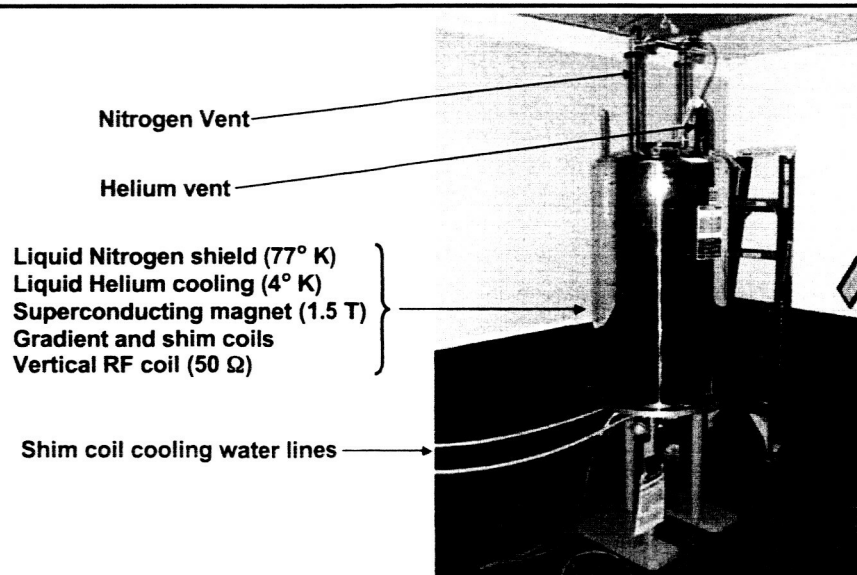
Control system and electronics



5



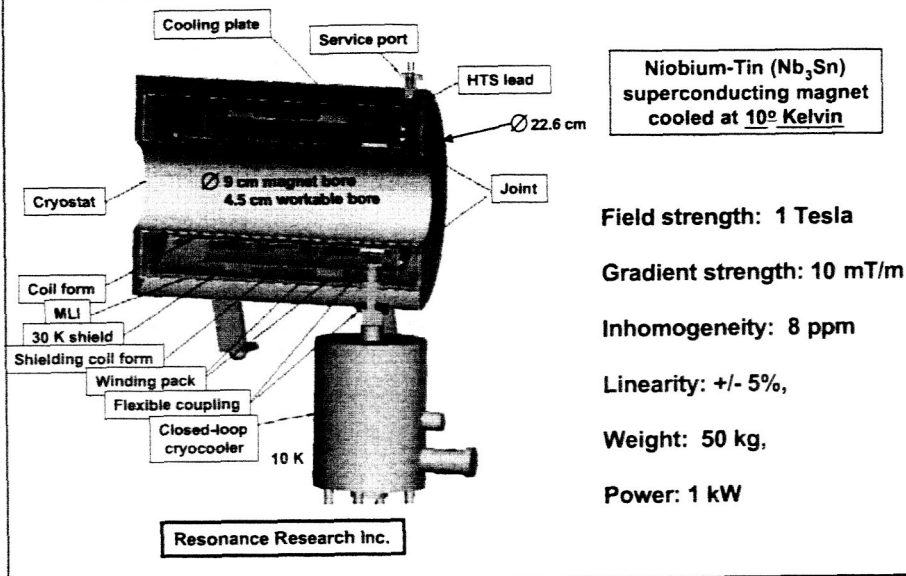
Current superconducting magnet



6



Superconducting magnet design



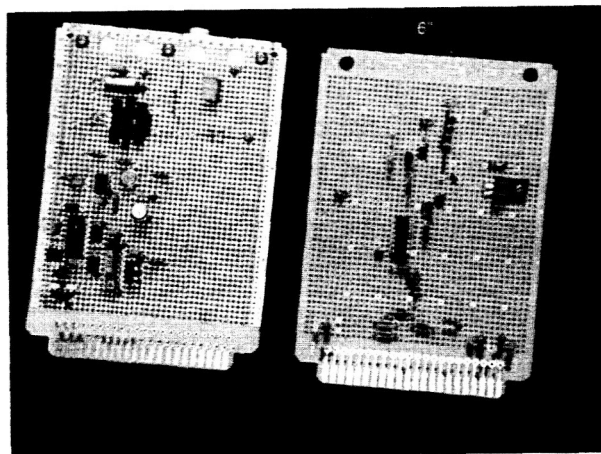
7



New RF amplifier



- Built on 3 circuit cards
 - 6"x8"x1"
- Efficiency
 - RF+Modulator: 80%
 - Power Supply: 75%
- Class E RF Amp
- Class S Modulator
- Average power : 1W



8



Average power consumption



	Commercial unit	SQMRI unit
RF amplifier	350 W	1 W
Gradient amplifiers	2000 W	50 W
Computer and console	200 W	100 W
Cooling system	10 W	1000 W
TOTAL	2560 W	1151 W

9



Images from baseline system



Object:

- cherry tomato
- 3.5 cm diameter

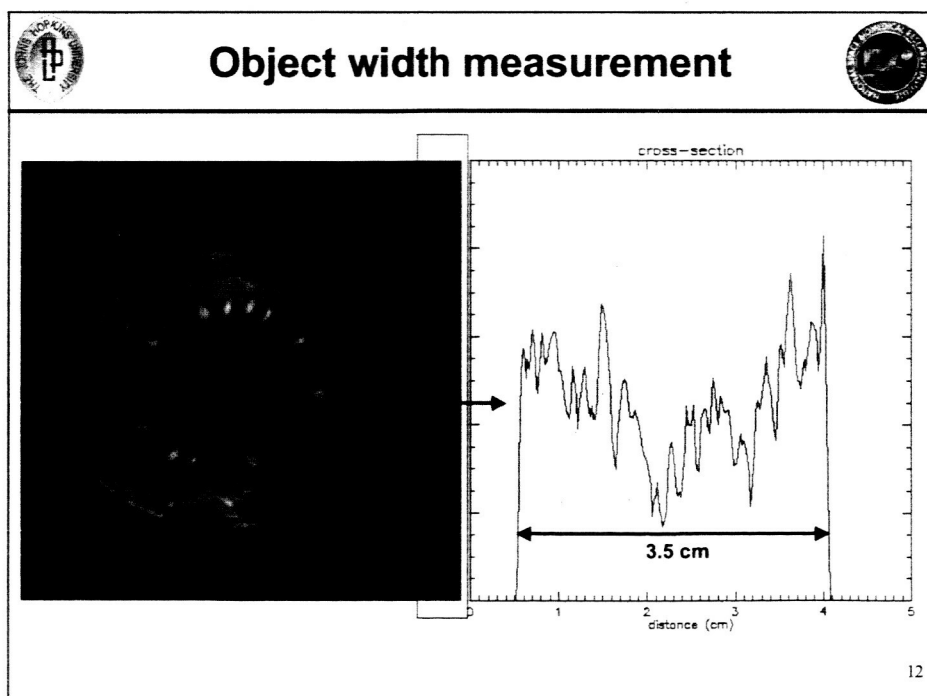
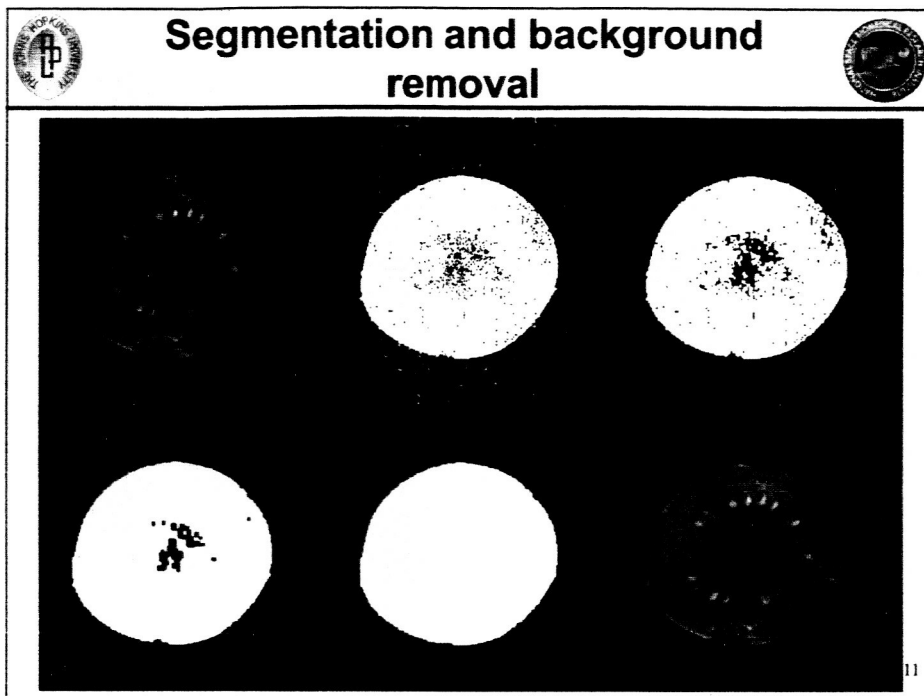
Imaging:

- spin echo
- 20 slices
- 256x256

Resolution:

0.2 mm







Example of planned software steps in operational module



The software of the MRI system will be designed to facilitate and automate the analysis and interpretation of images by astronauts. For example, in the assessment of bone loss, several image processing functions must be accomplished reliably to obtain accurate results:

1. To ensure the same 3D orientation in images.
2. To analyze the same part of the same bone in each image.
3. To determine the edge of the bone consistently and accurately in each image.
4. To ensure thickness measurement along a line perpendicular to the bone edge.
5. To issue a warning if a measurement is degraded, due to image noise or artifacts.
6. To record and annotate the measurements in a correct, informative and user-friendly manner.
7. To analyze the thickness measurements and obtain a reliable interpretation of the study.

The operational software module will include such functions to allow the astronauts to obtain bone or muscle loss measurements in a fully automated manner.

13

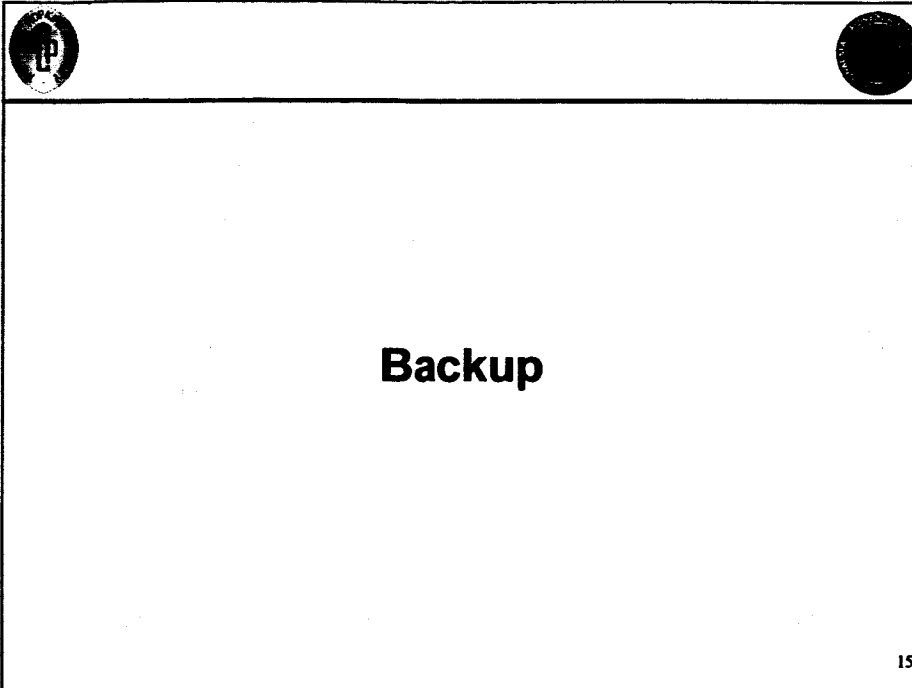


Conclusion



- Power-efficient amplifier electronics contributed significantly.
- Baseline MRI system provides a good starting point for development.
- Closed-loop cooling system will consume 87% of the power.
- Advances in cooling systems and superconducting magnet coil technologies may enable 10 cm bore size in the near future.
- The potential contributions of the Space Qualified MRI system are likely to be realized in two ways:
 1. By assisting NSBRI research teams in imaging and measuring anatomical and physiological changes in their subjects.
 2. By allowing astronauts to monitor structural and functional changes in space.

14



NASA Advanced Cryocooler Technology Development Program (ACTDP)

New program for cryogen-free, lightweight, low-power mechanical cryocoolers to support three NASA missions with similar cooling requirements, at 6 K and 18 K.

Missions	Cryocooler Requirements	Cryocooler Manufacturers	Weight	Input Power
1. Next Generation Space Telescope 5 meter telescope at 1.5 million km from Earth, will replace the Hubble ST	40 mW @ 6 K for detectors 250 mW @ 18 K for mirrors and optical components	Ball Aerospace	27 kg	150 W
2. Terrestrial Planet Finder Four 3.5 meter telescopes flying in formation, will search for earth-like planets		Lockheed Martin	26 kg	208 W
3. Constellation – X A group of 4 spacecraft, each carrying two X-ray telescopes, will explore black holes and galaxy formation		TRW Northrop Grumman	17 kg	207 W

Space Qualified MRI
Stage 1: 2000 mW @ 30 K,
Stage 2: 50 mW @ 10 K
<35 kg
<1000 W

16



Improved Bubble Detection for EVA

**Status: Ongoing,
Year 1 of
Continuation**

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Patrick J. Magari, Ph.D.,
Marc Kenton, Ph.D.
Darin Knaus, Ph.D.
Creare, Inc.
P.O. Box 71
Hanover, NH 03755
pjm@creare.com



Creare

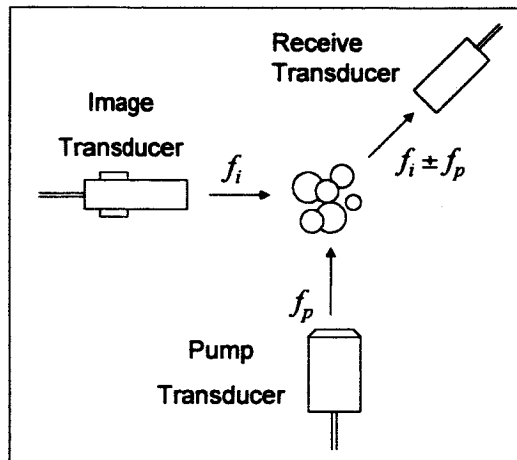
Bubble Detection-*Project goal*



- This project develops and validates new bubble detection technology (dual frequency ultrasound), which offers the potential for quantitative, real-time detection and sizing of bubbles in both tissue and blood.
- The technology can detect, locate and size bubbles from 1-200 microns in diameter, allowing for both extra- and intra- vascular use.
- The goal is to improve the safety of EVA.
- ISS construction and Mars exploration will require extensive and unprecedented extravehicular activity (EVA)

Bubble Detection-*Technical Approach*

In the actual machine, the image transducer both sends and receives (i.e. no separate receive transducer is needed)



Bubble Detection-*Tasks/Milestones*

- **Signals consistent with bubbles have been detected in both tissue and blood.**
- **Histograms of bubble sizes have been constructed during decompression stress.**
- **Work to calibrate the device is underway.**
- **The ability to combine bubble detection with 2-D ultrasound has been demonstrated.**

Bubble Detection-*Accomplishments*

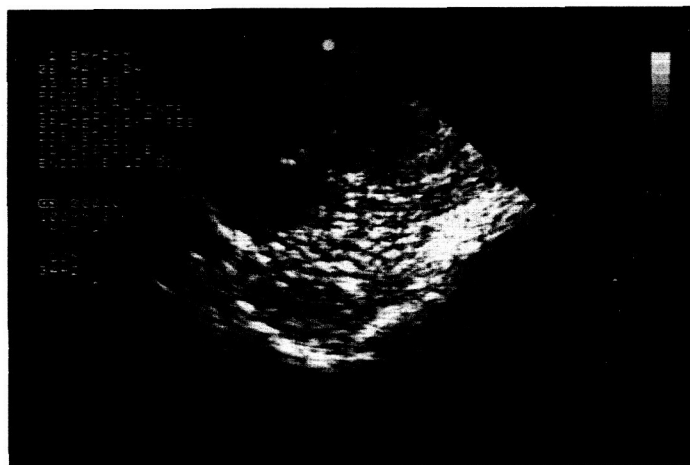


- Constructed histograms of bubble sizes *in-vivo*
- Suggestion that histograms can change with interventions (Oxycyte)
- *In-vitro* calibration ongoing
- Evaluating factors that influence bubble detection and sizing (transducer harmonics, bubbles at half or double resonant size)
- Digital implementation of device completed

Bubble Detection-*Accomplishments*



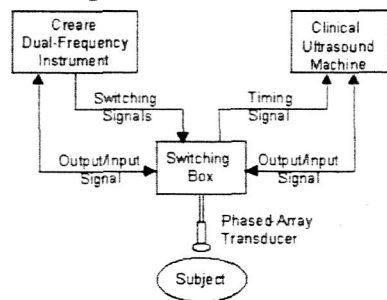
Bubble
detection
during
DCS.



Bubble Detection-*Accomplishments*



- Create bubble detection instrument integrated with HP Sonos 1000
- Provides seamless, automated, simultaneous operation of both instruments
- Complete *in-vitro* and *in-vivo* validation ongoing



Bubble Detection-*Next steps*



- Application of new technology to extra-vascular applications (tissue bubbles)
- Intravascular bubble size calibration and application to decompression stress.
- Three critical factors for research/operational use of device:
 - Depth scanning for tissue bubble location.
 - Incorporation with 2-D ultrasound for accurate targeting in both intravascular and extravascular applications.
 - Miniaturization



AMPDXA-GCS NSBRI Technology Team Retreat 9 September 2004

Harry K. Charles, Jr., Ph.D.

**The Johns Hopkins University
Applied Physics Laboratory**

AMPDXA-GCS
Technology Development Team Retreat (9/9/04)-1



AMPDXA-GCS



- **Project Title:** Ground-Based Measurement of Bone Loss in Astronauts Using the AMPDXA Ground-Based Clinical System
- **Name/Address**
Performing Institution: The Johns Hopkins University
Applied Physics Laboratory
11100 Johns Hopkins Road
Laurel, Maryland 20723-6099
- **PI/Contact Information:** Harry K. Charles, Jr., Ph.D.
Head, Technical Services Department
Phone: (240) 228-8050
Fax: (240) 228-6119
Email: harry.charles@jhuapl.com
- **Project Status:** New FY04 (September '04)

AMPDXA-GCS
Technology Development Team Retreat (9/9/04)-2



Project Goals



- **Design and build an AMPDXA Ground-Based Clinical System (AMPDXA-GCS).**
 - Human qualified with full body scans.
 - Pre- and post-flight assessment of astronauts.
- **Validate the Safety and Utility of AMPDXA-GCS Technology.**
 - Although based on previously NSBRI funded AMPDXA Laboratory Test Bed (LTB) and Human Test Bed (HTB), it will be necessary to re-evaluate the AMPDXA in the GCS configuration because of the technology and protocol changes.
- **Address technical challenges to future AMPDXA use in space.**
 - Compact, pulsed, high efficiency power source with high speed energy switching.
 - Advanced high efficiency, energy switching self-cooled x-ray source.
 - Stowable Scanner Configuration.
- **Transfer AMPDXA-GCS Technology to Commercial Clinical Systems.**
 - Commercialization Efforts.
 - Utility in ground-based research and clinical diagnosis.

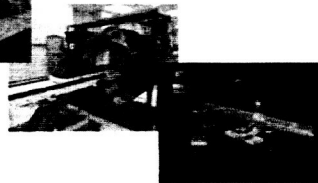
AMPDXA-GCS
Technology Development Team Retreat (9/9/04)-3



Technical Approach / Methodology



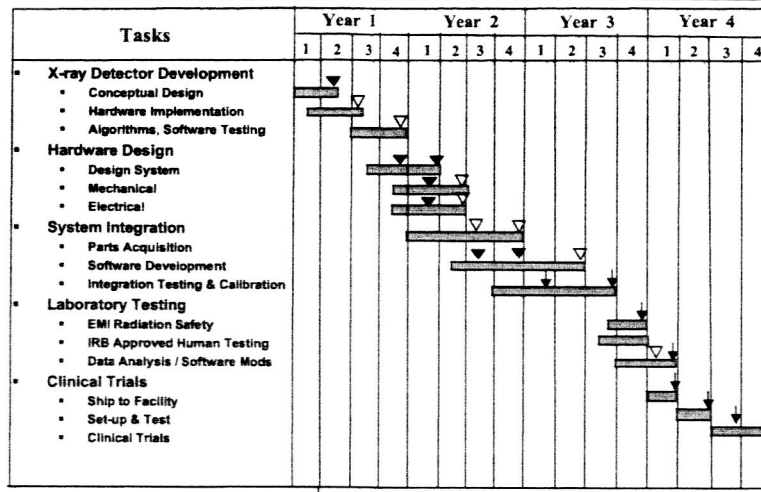
- **AMPDXA (1997-2004). Three-Phase Instrument Development.**
 - **Laboratory Test Bed (LTB)**
 - Operational almost 5 years
 - Fundamental concept and principal validation
 - **Human Test Bed (HTB)**
 - Operational for 2+ years
 - Human Images
 - **Flight Prototype Design**
 - Conceptual Design
 - Weight Budget
 - Critical Hardware Components Identified
- **AMPDXA-GCS (2004-2008) Two-Phase Instrument Development.**
 - **Verify new scan principles (LTB).**
 - Fixed scan angles (0° , $\pm 15^\circ$)
 - Dual Traverse
 - **Construction AMPDXA-GCS**
 - Design
 - Fabrication
 - Test & Evaluation



AMPDXA-GCS
Technology Development Team Retreat (9/9/04)-4



Schedule and Milestones AMPDXA-GCS



▼ Design Reviews, ▽ Implementation Reviews, ↓ Data Review

AMPDXA-GCS

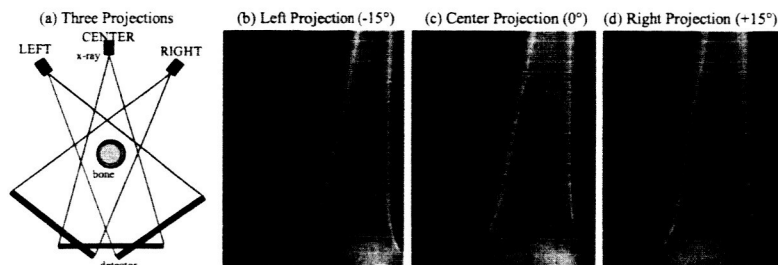
Technology Development Team Retreat (9/9/04)-5



Accomplishments (1)



- **Verified Detector-Source Geometry of the AMPDXA-GCS using the HTB (three fixed projection angles over a 30° arc (0°, +/-15°) rather than the 90° arc for the original AMPDXA).**



AMPDXA-GCS

Technology Development Team Retreat (9/9/04)-6



Accomplishments (2)



- AMPDXA-GCS Configuration (on HTB) – Structural Measurements (AMPDXA measurements compared to actual dimension on artificial bone cylinders)

Parameter	AMPDXA	Actual	% Difference
Diameter (cm)	2.17	2.20	-1.36
Cross-sectional Area (cm ²)	2.28	2.26	+0.88
Moment of Inertia, I _x (cm ⁴)	0.96 ¹⁾	0.96	-0.53
Moment of Inertia, I _y (cm ⁴)	0.93	0.96	-3.13

¹⁾0.955 to the next decimal place

- AMPDXA-GCS Configuration (on HTB) – Repeatability Measurements (5 sequential measurements)

Parameter	Standard Deviations
Diameter (cm)	±0.019
Cross-sectional Area (cm ²)	±0.012
Moment of Inertia, I _x (cm ⁴)	±0.021
Moment of Inertia, I _y (cm ⁴)	±0.033

AMPDXA-GCS
Technology Development Team Retreat (9/9/04)-7



Next Steps



- Near Term
 - Validate program, funding and staff availability plans.
 - Perform Conceptual Design of new X-ray Source/Detector System.
 - Modify LTB for new detector configuration and develop operational and analysis protocols.
 - Review current analysis software and modify as needed for new source/detector system.
 - Develop data management plan and HIPAA* compliance plan.
 - Start overall design of AMPDXA-GCS.
- On-going
 - Continue commercialization efforts.

*Health Insurance Portability and Accountability Act

AMPDXA-GCS
Technology Development Team Retreat (9/9/04)-8



2004 NSBRI Technology Team Review



Combined Ion and Neutron Spectrometer for Space Applications (CINS)

R. H. Maurer, D. R. Roth, J. O. Goldsten,
D. K. Haggerty and Cary Zeitlin

The Johns Hopkins University Applied Physics Laboratory
11100 Johns Hopkins Road, Laurel MD 20723

Richard.maurer@jhuapl.edu

New FY 04 Project

September 2004



Project Goal



- The Combined Ion and Neutron Spectrometer (CINS) will combine an improved model of the MARIE instrument on the Mars Odyssey mission with the previously developed NSBRI neutron spectrometer to monitor the complete radiation environment
- After fabrication and calibration are complete, it will be used in ground based accelerator experiments to determine energy spectra which will be compared with the responses of LET spectrometers and dosimeters.
 - The dose or dose equivalent calculated from the CINS energy spectra will be compared with the measured LET or dose of TEPCs or dosimeters to ascertain the limitations in response of the latter devices.

September 2004



Technical Approach



- Create a charged particle detector stack system that improves the MARIE instrument flying on the Mars Odyssey mission.
 - > improve the dynamic range and data rate
 - > particle identification by species from protons to iron
 - > 12-14 silicon detectors including 1mm thick position sensitive detectors and 5mm thick deposited energy detectors
- Thoroughly evaluate and calibrate the Bicron 454 scintillator detector system for medium energy neutrons.
- Develop the instrument electronics design based on the Gamma Ray Neutron Spectrometer (GRNS) instrument for the MESSENGER mission.

September 2004



Tasks and Milestones



- Funding was finally received on August 18, 2004.
- The main tasks for the next six months are to get the detector systems for both the charged particles and neutrons designed and fabricated.
- Using GEANT 4 model the Oct. 2003 balloon flight data to deduce the most probable incident neutron spectrum outside the instrument box from the moderated spectrum detected inside.
- Compare our thick target neutron production data to that of late 1980s Los Alamos data for publication in Radiation Protection and Dosimetry.
- Write summary article on the NSBRI Neutron Spectrometer for the APL Technical Digest.

September 2004



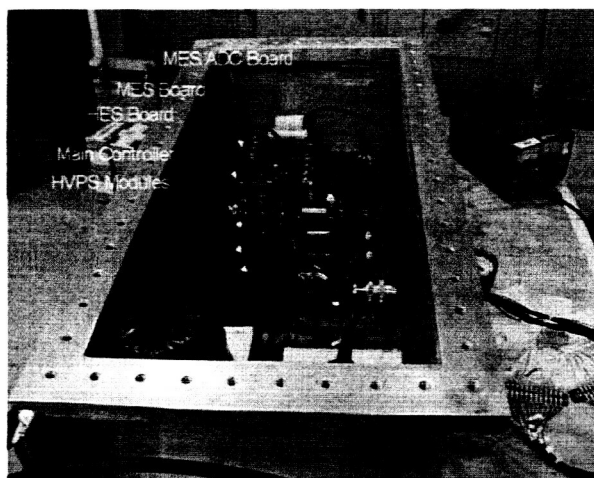
Accomplishments

- High altitude balloon flights (85,000 feet)
 - Qualification May 16, 2003 at Fort Sumner, NM
 - Flight October 9, 2003 from Fort Sumner, NM
 - Model effect of aluminum box, air inside box and CsI shield to deduce neutron spectra outside instrument from highly moderated detected spectrum using appropriate transport code.
- Support NASA spacecraft shielding materials research at ground based accelerators
 - 200 MeV protons on axis at IUCF November 2002
 - 500/350 MeV protons at TRIUMF September 2003
 - 200 MeV protons off beam axis at IUCF November 2003
 - Results presented at International Radiation shielding Conference on Madeira Island, Portugal in May 2004

September 2004



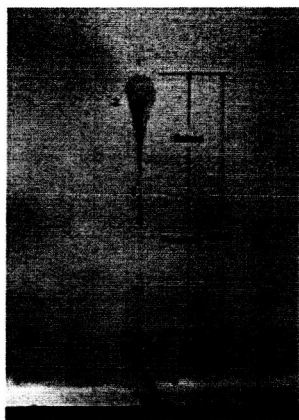
Balloon Flight Instrument 2003



September 2004



Balloon Launch 10/09/03, 0822 MDT

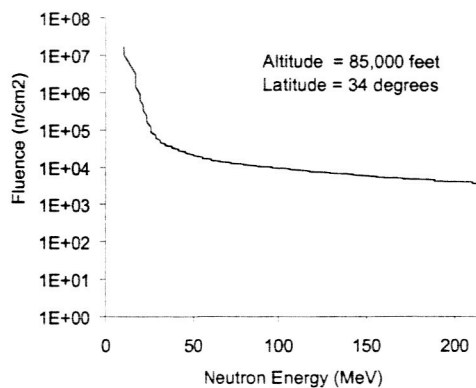


September 2004

1. Balloon Release Valve.
2. Balloon Fill Valves.
3. Balloon Release Ring.
4. Parachute Release Ring.
5. Payload.
6. Launch vehicle.



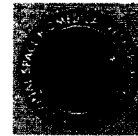
Preliminary Data: Uncorrected Balloon Flight Neutron Integral Energy Spectrum



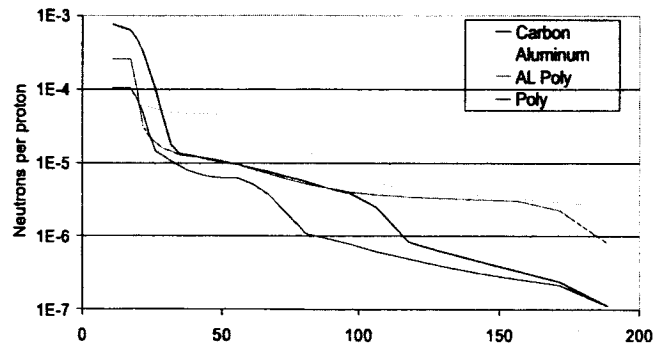
September 2004



Neutron Energy Spectra from Collisions of 200 MeV Protons with Thick Spacecraft Shielding Materials I



0 Degree Integral Neutron Energy Spectra



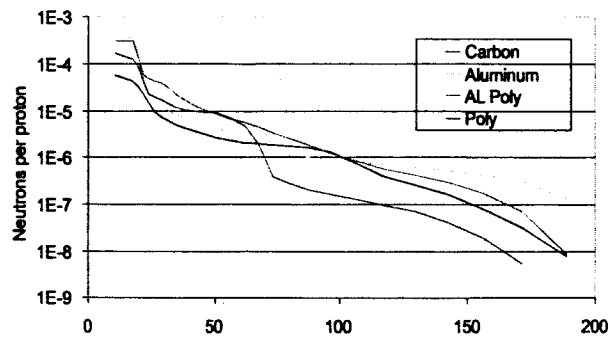
September 2004



Neutron Energy Spectra from Collisions of 200 MeV Protons with Thick Spacecraft Shielding Materials II



60 Degree Integral Neutron Energy Spectra



September 2004



Next Steps



1. In response to a request by NASA Marshall we submitted a proposal to supply a version of the NSBRI Neutron Spectrometer for the Deep Space Test Bed (NSTB) Antarctic balloon flights beginning in December 2005. The contract is in the sign off phase between APL and Marshall and we will be funded for \$243,000 to deliver the instrument in Sept. 2005.
2. We are starting the purchasing process for CINS detectors.
3. In collaboration with The University of California Berkeley, Lawrence Berkeley National Laboratory, Los Alamos National Laboratory, California Institute of Technology and NASA Johnson, Langley and Marshall we are submitting a proposal for a global Radiation Environment Measurement System (REMS) for the 2008 Lunar Reconnaissance Orbiter mission.

September 2004



Peer Reviewed Publications I

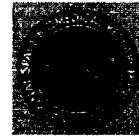


- Early shielding studies results: R. H. Maurer, D. R. Roth, J. D. Kinnison, T. M. Jordan, L. H. Heilbronn, J. Miller and C. J. Zeitlin, "Neutron Production from Polyethylene and Common Spacecraft Materials," IEEE Transactions on Nuclear Science **48**, 2029-2033, Dec. 2001
- Neutron detection with thick silicon detectors including the energy spectrum deconvolution method: J. D. Kinnison, R. H. Maurer, D. R. Roth and R. C. Haight, "High-Energy Neutron Spectroscopy with Thick Silicon Detectors," Radiation Research **159**, 154-160, Feb. 2003
- R. H. Maurer et al, "MArtian Neutron Energy Spectrometer (MANES): an instrument for the Mars 2003 Lander" Acta Astronautica **52**, 405-410, Feb. 2003

September 2004



Peer Reviewed Publications II



- R.H. Maurer, J. D. Kinnison, D. R. Roth, "Neutron Energy Spectra from 200 MeV Proton Interaction with Spacecraft Materials," results from November 2002 IUCF accelerator experiments; presented at the Annual IEEE Radiation Effects Conference (NSREC) in July 2003
- J. D. Kinnison, R. H. Maurer, D. R. Roth, P. J. McNulty and W. G. Abdel-Kader, "Neutron-Induced Pion Production in Silicon-Based Circuits," presented at the Annual IEEE Radiation Effects Conference (NSREC) in July 2003 and published: IEEE Transactions on Nuclear Science **50**, 2251-2255, Dec. 2003
- R.H. Maurer, J. D. Kinnison and D. R. Roth, "Neutron Production from 200-500 MeV Proton interaction with spacecraft Materials", ICRS 10/RPS 2004, Madeira Island, Portugal, May 2004

September 2004

MicroDosimeter iNstrument (MIDN)

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United States Naval Academy
Aerospace Engineering Department (Stop 11B)
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F. A. Cucinotta PhD, NASA/JSC

M. E. Nelson PhD, USNA

M. Zaider PhD, Sloan-Kettering

J. F. Zeigler PhD, USNA

Year One of New Project, 2004-2008

Slide 1 Title

MIDN GOALS

- Develop a small, compact, and portable, flight qualifiable, solid-state, real-time microdosimeter to measure quantitative information on the dose and dose distribution of energy deposited in silicon cells of tissue size and by inference in tissue.
- Analyze MIDN data from radiation beam experiments and compare with radiation transport codes to provide quantitative information on the radiation environment, potential risk, and the accuracy of the codes to correctly calculate energy-deposition spectra.
- Correlate MIDN data from radiation beam experiments with radiation transport codes to determine the effectiveness of selected materials to minimize the total risk from primary and secondary radiation.

Slide 2 Goals

MIDN TECHNICAL APPROACH

- Instrumentation
 - Develop engineering model and carry out initial electrical and radiation tests
 - Continue to refine instrument
 - Construct protoflight unit
- Testing
 - Preliminary tests at USNA
 - Tests at Brookhaven and other possible opportunities
- Simulations
 - Refine simulations
 - Carry out simulation to interpret test results
- Supplemental Project
 - Flight test of preliminary instrument in September 2006 on MidSTAR-I

Slide 3 Approach

MIDN TECHNICAL MILESTONES 1/2

Preliminary Results

- Complete test set-up with MCA
- Specification of sensor using pulsed input
- Preliminary results with AM241 alpha source

August 2004 – July 2005

- Develop engineering model and carry out preliminary tests
- Develop test instrument and carry out preliminary electrical and engineering tests at USNA
- Develop an integrated test and calibration plan
- Carry out initial simulations

Slide 4 Milestones 1/2

MIDN TECHNICAL MILESTONES 2/2

August 2005 – July 2006

- Complete final test instrument
- Tests at Nuclear Engineering Laboratory at USNA
- Two tests at Brookhaven
- Simulation of test results

August 2006 - July 2007

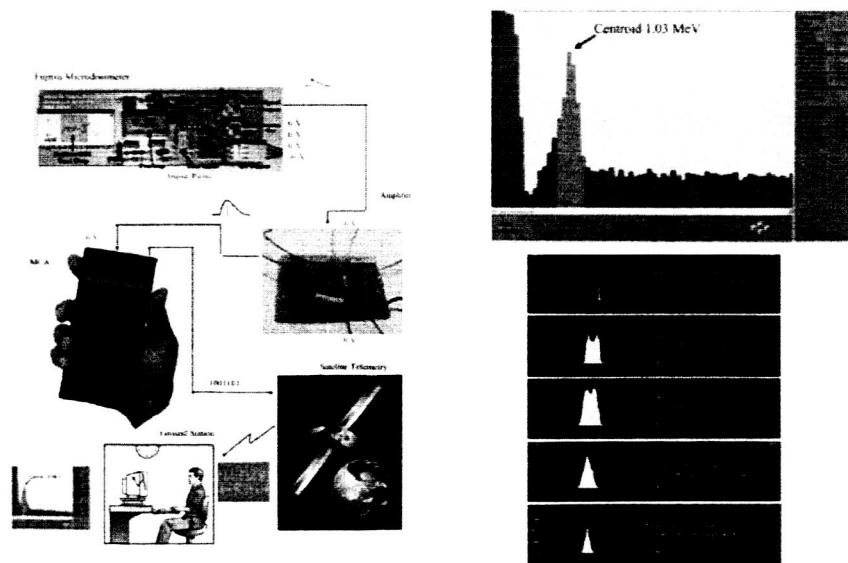
- Refine instrument
- Tests and calibration at Brookhaven
- Simulations of test results

August 2007 - July 2008

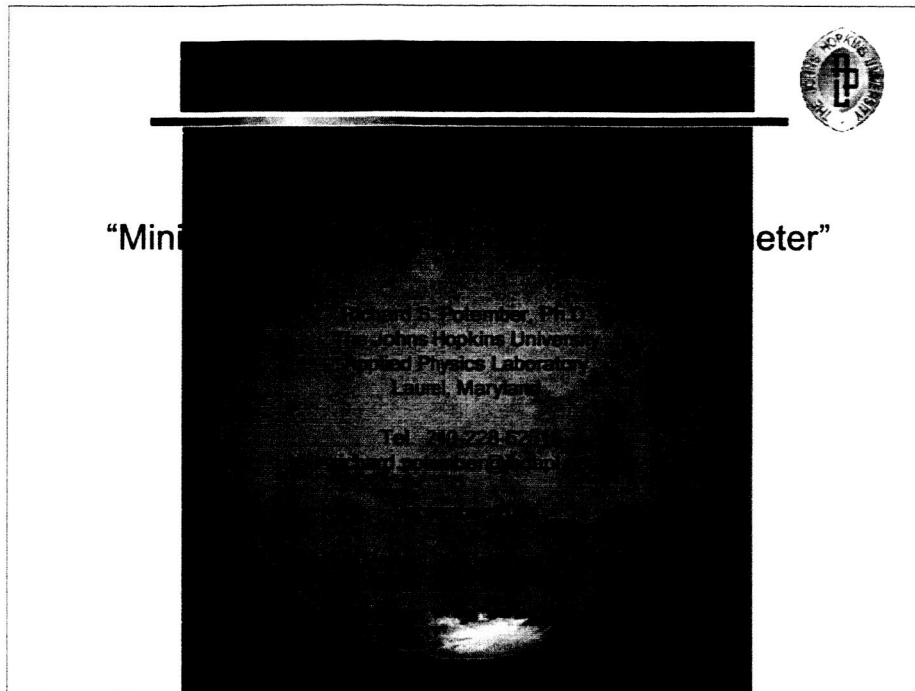
- Complete test instrument
- Build protoflight unit
- Final calibration at Brookhaven
- Simulation of test results

Slide 5 Milestones 2/2

MIDN PRELIMINARY RESULTS TEST SET-UP and RESULTS



Slide 6 Preliminary Results



Project Goal / Hypothesis

The goal of this project is to develop the necessary protocols and procedures to apply the APL “Miniature Time-of-Flight Mass Spectrometer” (TOFMS) in partnership with NSBRI/NASA for use in space. This compact medical diagnostic system will provide semi-autonomous patient monitoring systems with low false positive alarm rates. The instrument will be compatible with expert medical systems and telemedicine procedures.

The **hypothesis** of this research is that a miniature time-of-flight mass spectrometer can be used to monitor, in “real time,” a wide array of human physiological functions (**Biomarkers**) required to protect humans during spaceflight.

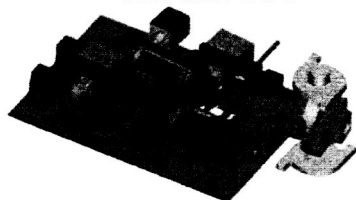
The time-of-flight mass spectrometer can also be used to evaluate the effectiveness of applied **Countermeasures** to the adverse effects on spaceflight.

The miniature time-of-flight mass spectrometer can also be used for **Environmental Monitoring** of the spacecraft (harmful bacteria, fungi, etc.).

Two Embodiments

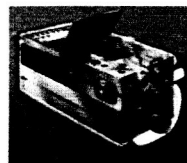


Aerosol-TOF



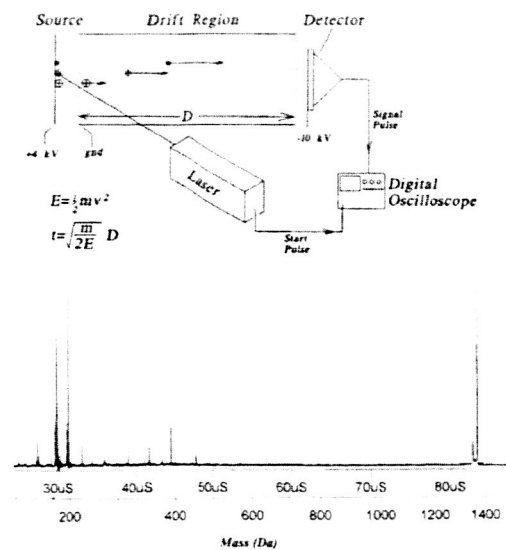
- Aerosol Sampling
- Unattended, Continuous, Long-Term Operation
 - Automated Sample Collection/Processing
 - MALDI-TOF Mass Spectrometry
 - Automated Detection Processing
- Rapid, High Reliability Detection
- Size, Weight, Power Appropriate for Area Protection

Clinical-TOF



- Manual Samples (Swipes, Swabs, Powder, Liquid)
- Person-in-the-Loop Operation
 - Automated Sample Processing
 - Manual Sample Introduction
 - MALDI-TOF Mass Spectrometry
 - Automated Detection Processing
- Rapid, High Reliability Detection
- Size, Weight, Power Appropriate for Response Teams

Time-of-Flight Mass Spectrometry



Matrix-Assisted Laser Desorption Ionization

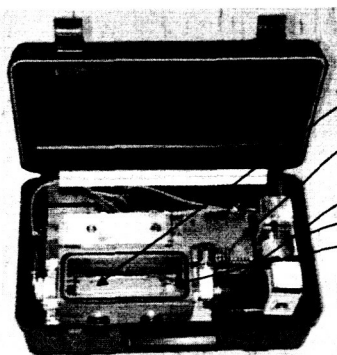


- MALDI Advantages
 - practical mass range up to 300,000Da
 - Low femtomole to low picomole sensitivity
 - Soft ionization technique
 - High tolerance of salts and buffers
 - Suitable for the analysis of complex mixtures

Portable MALDI TOF Detector



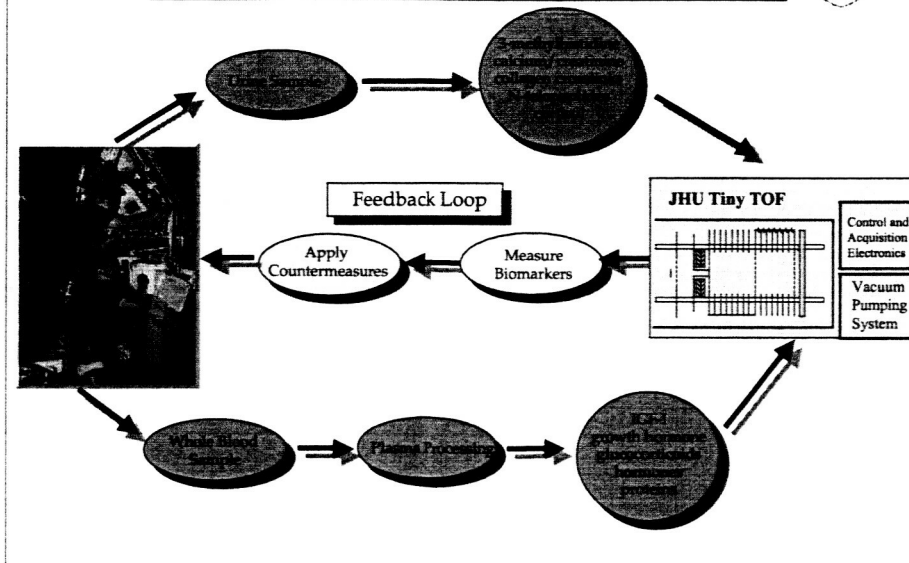
Demonstrates that all components are compatible with initial portable configuration



Unique Features

- Curved field, flexible-circuit reflectron
- Fast compact nitrogen laser, fiber optic beam delivery
- Gridless high field source, flat sample/tape interface
- State of the art miniature vacuum system
- Internal High Voltage DC converters

Concept For Sampling and Analysis of Biomarkers Using JHU Tiny TOF-MS



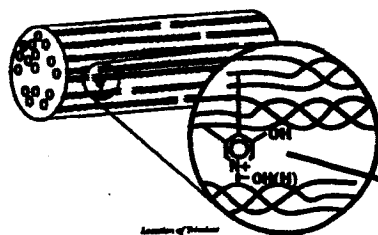
Bone Demineralization and Calcium Metabolism (J. Shapiro, M.D., Team Leader)



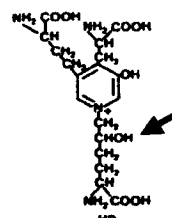
Medical Problem

- Skylab and Russian crews demonstrated 1.0-1.6% /month mean losses of bone mass from the spine, femur neck, and pelvis increase risk of fracture
- Alterations in skeletal metabolism pose substantial risks as mission duration is extended. Concern is lack of evidence that bone loss is reversible on return to earth
- To date, major countermeasures to decrease negative skeletal effects of space flight include weight loading exercises or artificial gravity regimes not effective preservation of skeletal mass

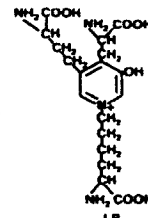
Trivalent Hydroxypyridinium Crosslinks Anchored to Type I Collagen Peptides In Bone Matrix



Location of Trivalent Hydroxypyridinium Crosslinks in Collagen Fibrils



MW = 429



MW = 413

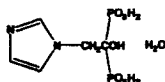
Zoledronate: a Countermeasure to Bone Loss



Milestone Year III:

We have completed our investigation to detect Zoledronate by MALDI-TOF. Zoledronate is a potent inhibitor of bone resorption.

For the treatment of benign bone disease zoledronate is given as a single i.v. dose of 5 mg, about 60% of the dose rapidly binds to bone and remains there for a very long time, the remaining 40% is rapidly excreted unchanged in the urine.



The physicochemical behavior of the molecule is dominated by the highly charged, hydrophilic phosphonic acid residues and there are also problems with the various protonation states of the PO_3 groups, and binding of cations to the zoledronate anion.

Researchers in Novartis (maker of zoledronate) have confirmed our results and they have informed us that they have tried for several years to develop a sensitive ionization mass spec assay for zoledronate in urine and plasma.

Based on our results and on our discussions with Novartis, we believe that we can best monitor the bone loss and the countermeasure effects of zoledronate by monitoring levels of trivalent hydroxypyridinium crosslinks and creatinine.

TOF Mass Spectrometry to Monitor Human Bone Resorption Using Type-I Collagen Cross-linked N-telopeptides in Urine



We have demonstrated that Cross-linked N-telopeptides of type-I bone collagen (NTx) can be measured using the TOF Mass Spectrometry.

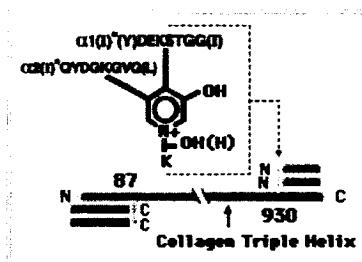
Due to its unique peptide sequence and cross-linked structure, the NTx molecule is a better indicator of bone resorption compared to urinary hydroxyproline or the free pyridinoline cross-links.

The NTx molecule resists further degradation, and is very stable in human urine.

We are investigating the time of flight mass spectrometer as a diagnostic tool to measure human bone resorption rates, utilizing N-telopeptides in urine as the test specimen.

Reference: Hanson DA, Weis MAE, Bollen AM, Maslan SH, Singer FR, Eyre DR. A Specific Immunoassay for Monitoring Human Bone Resorption: Quantitation of Type-I Collagen Cross-linked N-telopeptides in Urine. *J Bone Min Res.* 1992;7: 1251-1258.

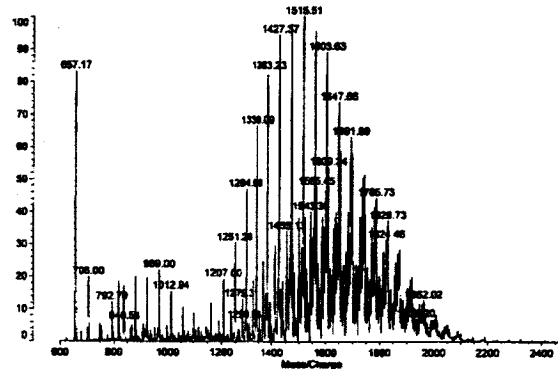
Intermolecular Cross-linked N-telopeptides Type I Collagen



- Elevated levels of NTx indicate elevated human bone resorption.
- Generation of NTx molecule is mediated by osteoclasts on bone and is found in urine as a stale end-product of degradation.

Reference Range:
24 Hr. Urine (25 ml aliquot)
15-150 micromoles/mol creatinine

TOFMS of Urinary Cross-linked N-Telopeptides Type I Collagen (1nM BCE Calibrator)



BCE = bone collagen equiv.

Assessment of Circadian Status Using the TOF Miniature Mass Spectrometer



Milestone: Year III

We are currently analyzing urine samples from Dr. Kenneth P. Wright Jr. for the excretion of the melatonin metabolite, 6-sulphatoxymelatonin.

The development of online methods for monitoring and assessing the status of circadian organization is one of the five primary themes for the Human Performance, Sleep and Chronobiology Team.

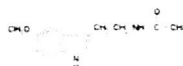
We are currently evaluating melatonin metabolite, 6-sulphatoxymelatonin in spiked buffered solutions using the sample protocol methods we developed in year II and we will complete this milestone by evaluating the melatonin metabolite, 6-sulphatoxymelatonin in urine samples.

Technical Approach



Melatonin Analysis

Melatonin (M.W. = 232.3g/mol) was characterized as a standard for analysis of urinary melatonin metabolite which could provide a safe and effective method to monitor generation of HO* in humans. Since melatonin exists in virtually all animal species and has a wide intracellular distribution and is readily detected non-invasively in urine it was of great importance to be able to measure the melatonin.



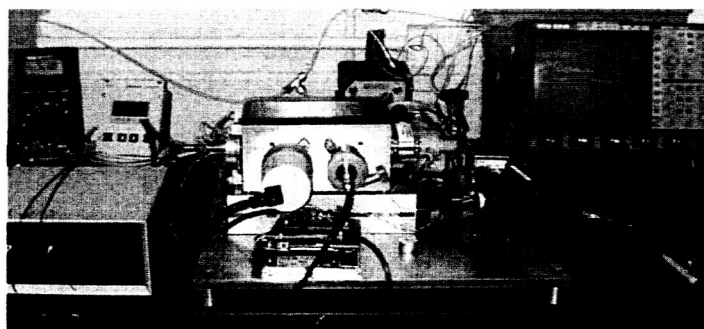
Chemical Structure IV of Melatonin (N-acetyl-5-methoxytryptamine.)

In our experiments, melatonin is being studied at a concentration of $1.27 \times 10^{-4}M$. A serial dilution was then performed and the series was analyzed with and without DHB matrix. MAS1 urine standard, a liquid assayed urinalysis control by Fisher, was spiked with different melatonin concentrations, mixed, cleaned up using ZipTips C18 and spotted onto the sample slide. Five replicates of each test were completed to assure accuracy.

Bench Top Prototype



Bench top unit at APL – Aug 2001



Features

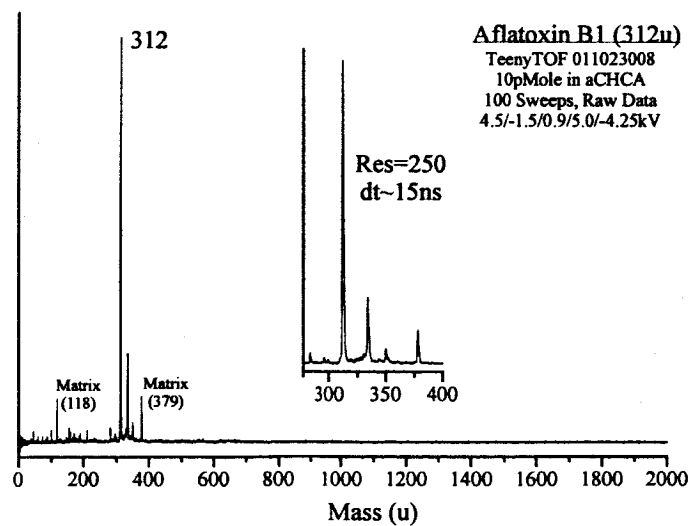
- Flex-circuit reflectron with integrated resistor network
- Gridless focusing ion source (High ion transmission)
- Fiber-Optic Laser Beam Delivery
- Micro-node MCP Detector

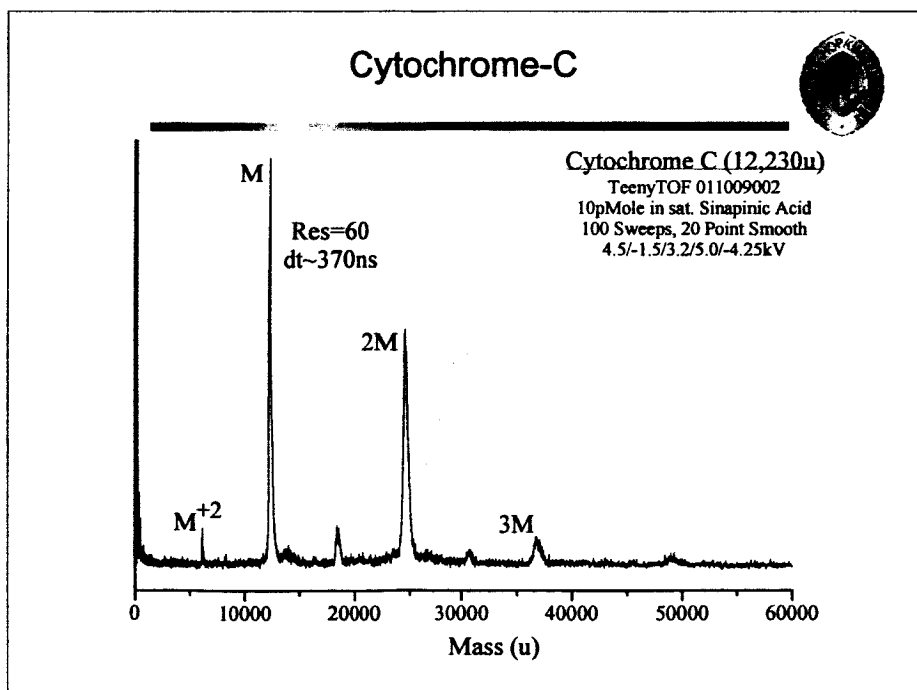
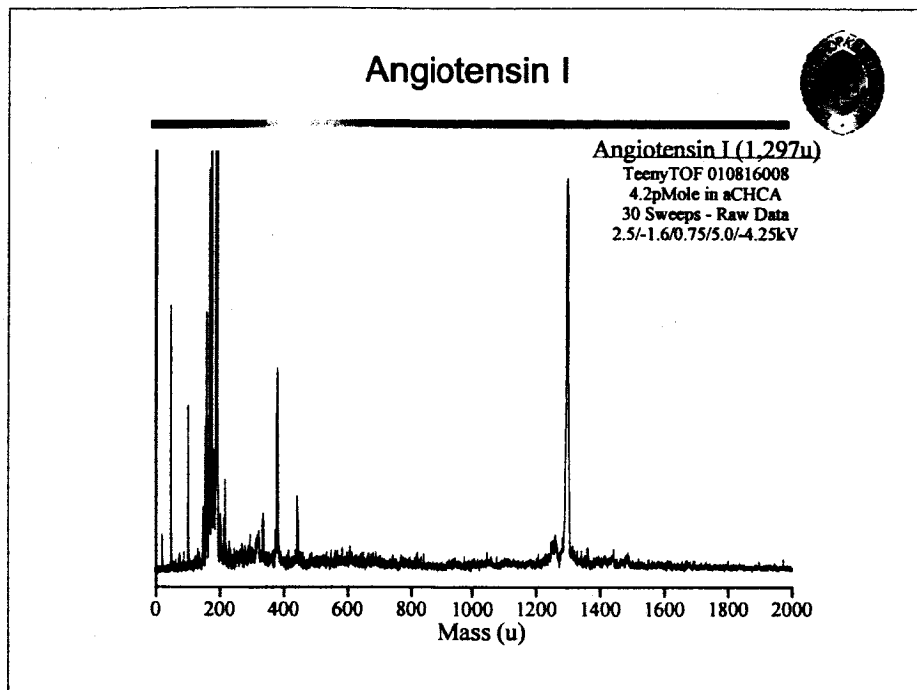
TOF System Components



Space-TOF-MS-System	
Analyzer	Flex circuit curved field reflectron, 5kV Integrated resistor chain.
Source	4.5kV gridless source, mini-gate valve, tape/slide interface
Detector	Custom low profile, coaxial, MCP, 6.5 kV post acceleration, reflectron detector
Vacuum	11 l/s Turbo-drag, diaphragm fore pump, coffin type chamber
Laser	LSI VSL337, N ₂ laser, fiber optic delivery (5ns, 140uJ, 28kW)
Power supplies	Manual 0-10kV COTS power supplies, 12/24 VDC
Digitizer	LeCroy 9354 Oscilloscope, 1GSa/s, GPIB
Processor	Panasonic Toughbook, USB-GPIB, LabTOF, software
Case	Pelican Case (base plate mounted for testing)

Alfatoxin B1



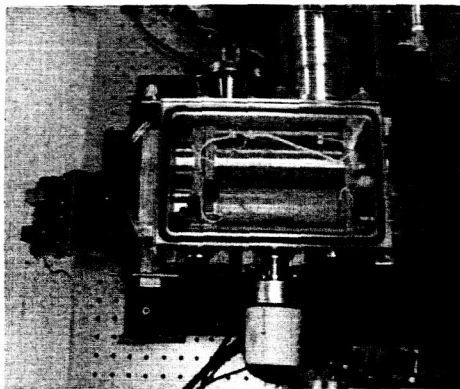


Next Generation Miniature TOF



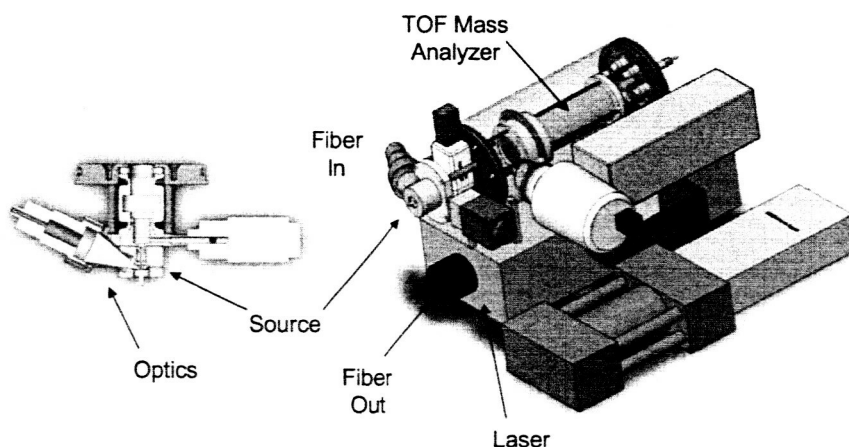
- Next generation includes many technical improvements.
 - Vacuum system – SS cylindrical chamber, source vacuum.
 - Redesigned optical system.
 - Double-sided detector (captures signals lost with standard detectors)
 - Compact data system – PXI modules, integrated electronics.
 - Extensive electronic control and feedback.
 - Packaging - one or two small cases or custom frame-work.
 - 30 Lb. with batteries for 30-60 minute operation.

Arrayed Time-of-Flight Analyzer Design

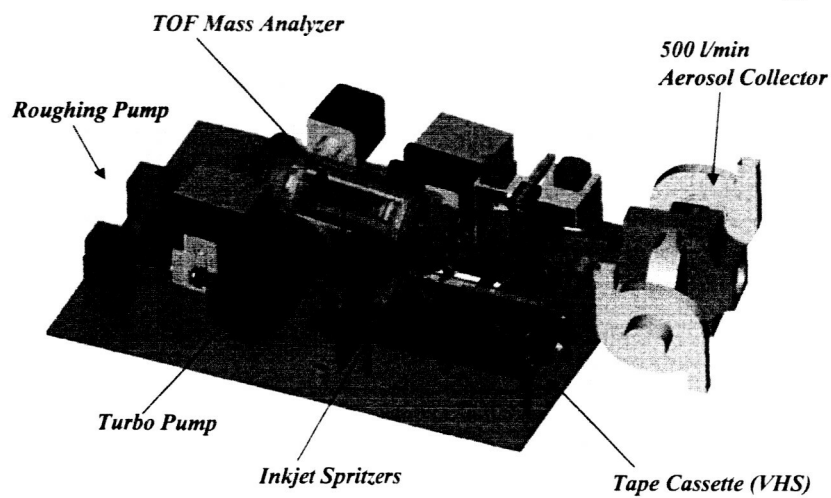


"In-line" array of miniature time-of-flight (TOF) mass spectrometers to produce a simple, rugged system capable of simultaneous data collection from samples. In addition to the increase in the data collection rates, simultaneous data collection enables a high degree of redundancy. This improves biomarker identification.

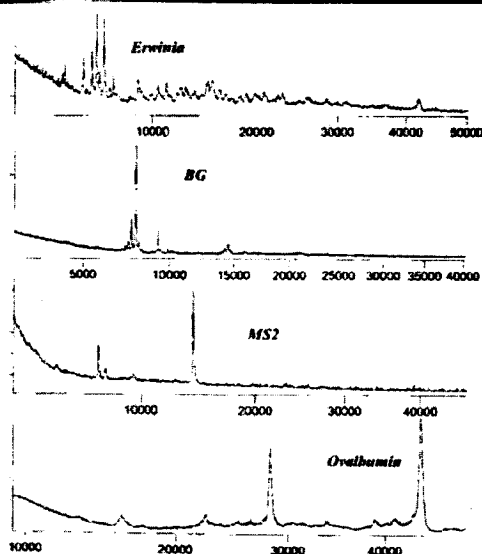
Design of Miniature Time-of-Flight Mass Spectrometer



Fully Automated Aerosol Collection TOF-MS



Dugway Simulants: BioTOF Array



RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2001, 15: 2130-2134

RCM

Detection of specific *Bacillus anthracis* spore biomarkers by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry



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¹Department of Microbiology and Molecular Genetics, Israel Institute for Biological Research, Ness-Ziona 74100, Israel

²Department of Analytical Chemistry, Israel Institute for Biological Research, Ness-Ziona 74100, Israel

³Department of Infectious Diseases, Israel Institute for Biological Research, Ness-Ziona 74100, Israel

Received 7 August 2001; Revised 20 September 2001; Accepted 21 September 2001

Table 1. Biomarkers of various *Bacillus* strains in linear mode

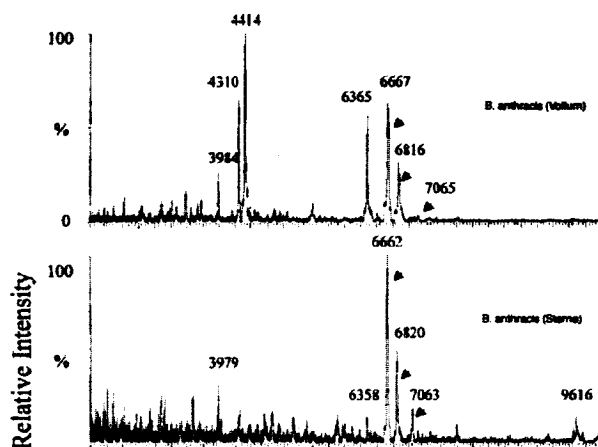
<i>Bacillus</i> strains	Mass markers* [M + H] ⁺						
<i>B. anthracis</i> , Vollum	4310	6365	6667	6816	7065		
<i>B. anthracis</i> , Sterne	4327	6358	6662	6820	7063	7744	9616
<i>B. anthracis</i> , 14185	4301	6357	6657	6811	7065		
<i>B. anthracis</i> , Δ14185	4304		6668	6825	7064		9621
<i>B. cereus</i>		6362	6698	6820	7065		9519
<i>B. thuringiensis</i> var. <i>thuringiensis</i>		6363	6698	6822	7067		9490
<i>B. thuringiensis</i> var. <i>israelensis</i>	4385	6446	6684	6851	7092		
<i>B. mycoides</i>	4971	5370	6027	6949	7395		
<i>B. subtilis</i> 168	4585	5802		6939			
<i>B. subtilis</i> BD104	4565	5821					9128
<i>B. licheniformis</i>	4818	5820	5892				
<i>B. subtilis</i> type <i>niger</i>	4410	5037	5582	6739	7056	7321	8876

* Masses (Da) were taken from representative spectra.

B. cereus group-specific biomarkers which have been repeatedly identified in at least five independent spore extractions within a mass range of ± 5 Da are underlined.

A limited number of proteinaceous biomarker peaks in the 4–7 kDa range can be reproducibly detected in *B. anthracis* strain extracts and they are not found in other *Bacillus* species spores.

Linear MALDI - TOF of *B. anthracis* Spore Extracts
(arrows indicate reproducible appearance of biomarkers)



Rapid Commun. Mass Spectrom. 2001; 15: 2110-2116

Next Steps



To design, build and test a fast, ground-based portable time-of-flight mass spectrometry instrument for evaluating astronaut biomarkers and countermeasures. This instrument can also be modified to evaluate Spacecraft Air Quality.

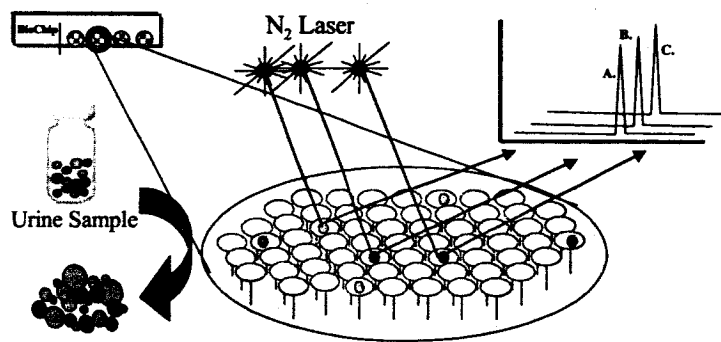
To develop sampling system and preparation techniques that enable the MALDI TOF mass spectrometer system to reliably detect, identify and quantify extremely low levels of chemical and biological substances in complex body fluids (urine, breath) with very low error rates.

Work with NASA scientists to demonstrate that the miniature time-of-flight mass spectrometer is an important diagnostic tool for space based applications.

Chemically Modified Matrix-Chip



- Urine sample goes *directly* onto the BioChip
- Biomarker molecules are captured on the chip (affinity capture)
- Surface-coated with Energy Absorbing Matrix (EAM)
- Matrix-Assisted Time-of-Flight/Mass Spectrometry





Scanning Confocal Acoustic Diagnostic System for Bone Quality

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Department of Biomedical Engineering
Stony Brook University
Email: yxqin@sunysb.edu Tel: 631-632-1481

Team:

Win Lin, Ph.D.: Software and control design
Barry Gruber, M.D.: Osteoporosis and clinical trial
Clinton Rubin, Ph.D.: Animal model
Yi Xia, M.S.: Surface mapping and imaging
Erik Mittra, Ph.D./M.D.: Micro-CT, mechanical
property

**Sponsor
NSBRI**

New York Advanced Center
for Biotechnology

Status:
Ongoing, Year 1 of
Continuation



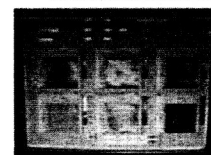
Objectives

1. Development of a portable, non-invasive, light weight, compact, easy to use scanning confocal acoustic diagnostic (SCAD) system for bone quality assessment in space.
2. Real-time trabecular bone imaging, capable of extracting both density and strength information from bone, and assessing risk of fracture
3. SCAD hardware and software developments for multiple skeletal sites, calcaneus, hip, wrist and knee. Technical aims: bone surface topology and automatic region of interests identification
4. Correlations between SCAD data and bone's structural (μ CT) and strength properties in human cadaver bones - for predicting bone quality
5. Clinical trial: osteoporotic population, bedrest, and pre- / post flight astronauts

Technical Approach / Methodology

(A) SCAD System Tech Development

1. Laboratory prototype for generating real-time ultrasound imaging in bone
 - SCAD for large ROI, i.e., hip
 - Confocal ultrasound hardware design
 - Imaging signal software interface
2. Improving accuracy SCAD imaging in deep tissues
 - Confocal bone surface mapping
 - Auto identification of trabecular ROI
3. Miniaturization of SCAD
 - Microprocessor circuit design
 - Computerization of data analysis, integration of BUA and UV
4. Clinical SCAD development
 - User friendly interface
 - Portable, compact, solid-state and mobile



Technical Approach / Methodology

(B) SCAD Testing and Validation

1. Testing, validation and data interpretation in human cadaver bones
 - Integrated confocal ultrasound parameters, BUA and UV
 - Bone's structural measurement (μ CT)
 - Mechanical stress testing, nanoindentation and bulk tissue strength
2. Clinical trials of SCAD
 - Osteoporotic patients
 - SCAD tests
 - DEXA measurement
 - Correlation



DEXA image

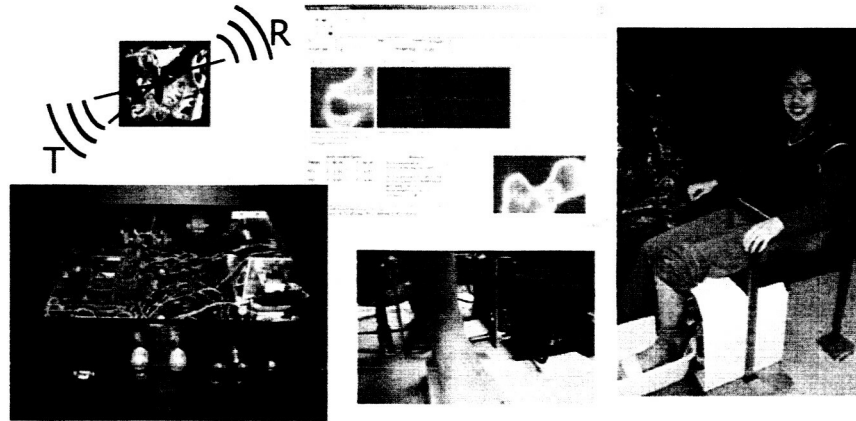
Tasks, Milestones and Schedule

Task	Year 1	Year 2	Year 3	Year 4
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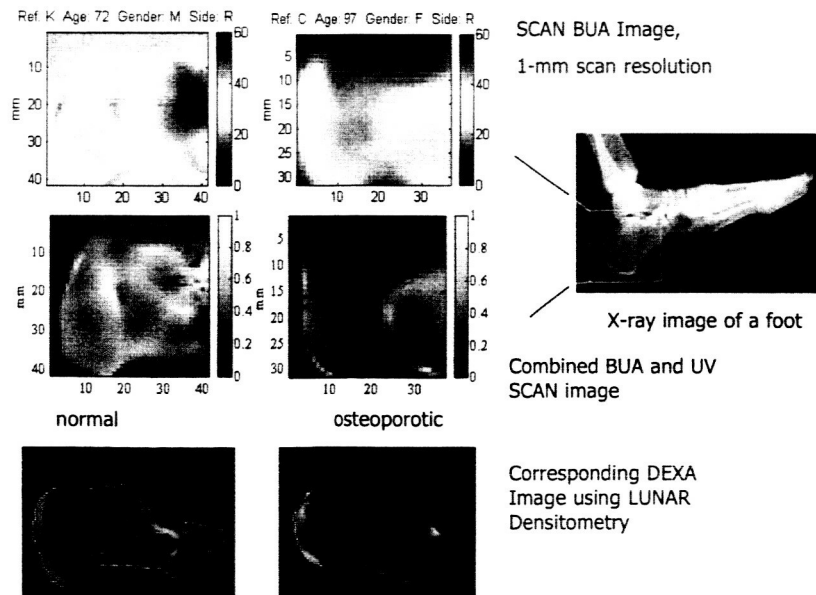
1. Laboratory prototype for generating real-time ultrasound imaging in bone
 - SCAD for large ROI, i.e., hip
 - Confocal ultrasound hardware design
 - Imaging signal software interface
2. Improving accuracy SCAD imaging in deep tissues
 - Confocal bone surface mapping
 - Auto identification of trabecular ROI
3. Miniaturization of SCAD
 - Microprocessor circuit design
 - Computerization of data analysis, integration of BUA and UV
4. Clinical SCAD development
 - User friendly interface
 - Portable, compact, solid-state and mobile
5. Testing and validation in human cadaver
6. Clinical trials of SCAD, bedrest, astronauts

SCAD – Accomplishment (1)

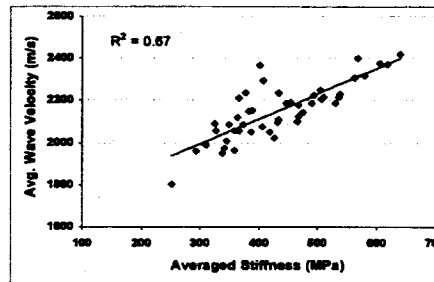
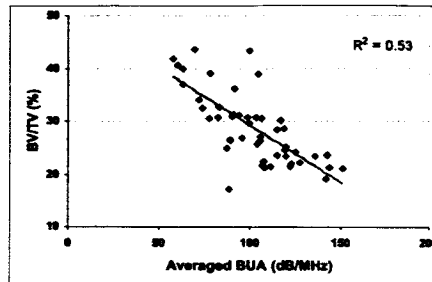
- Using ultrasound attenuation and velocity at acoustic focal points that reflect the density and stiffness of biomaterials
- Real-time mapping at the region of interest in bone 2-D or 3-D
- Reduce error from soft-tissue and cortical shell



SCAD – Accomplishment (2) – Test at human calcaneus



Correlations between bone volume fraction (BV/TV) and BUA, and between elastic modulus and UV

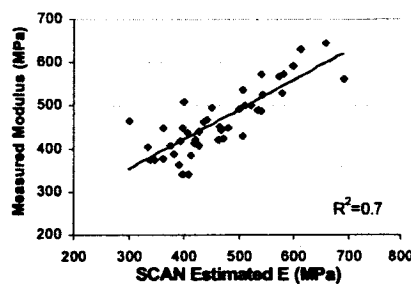


BUA ~ bone volume fraction

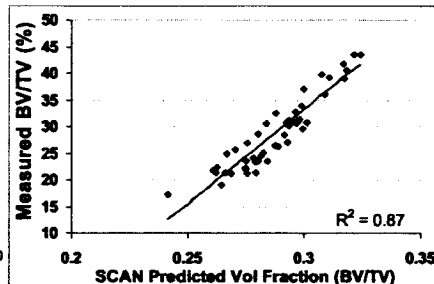
UV ~ bulk elastic modulus

- Average BV/TV is at approximately 35%
- Average UV is at approximately 2100 m/s

Prediction of Bone Mechanical Modulus and Bone Volume Fraction by *Combined* BUA and UV in a Model



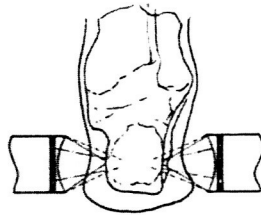
SCAN predicted E ~ Measured E



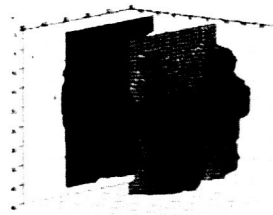
SCAN BV/TV ~ Measured BV/TV

- Mechanical strength -- 415 ± 100 MPa for bulk modulus
- Yield strength -- 16.5 ± 6.7 MPa
- Ultimate stress -- 18.6 ± 6.9 MPa
- Ultrasound scanning was capable of predicting the bone's quality parameters via multiple correlations
- Combined ultrasound parameters, BUA and UV yield significant correlation coefficients between ultrasound and measured bone quality.
 - Prediction of BV/TV and bone stiffness by ultrasound showed $R^2=0.87$ for BMD ($p<0.01$, left) and $R^2=0.70$ for stiffness ($p<0.01$, right) ($N=63$).

SCAD – Accomplishment (3) Work in Progress: Bone Surface Topology



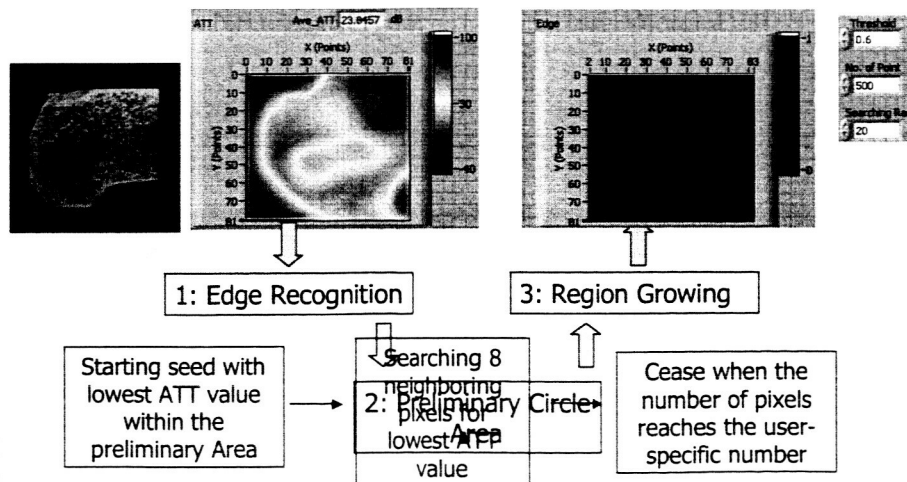
Bone surface detect



Surface topology

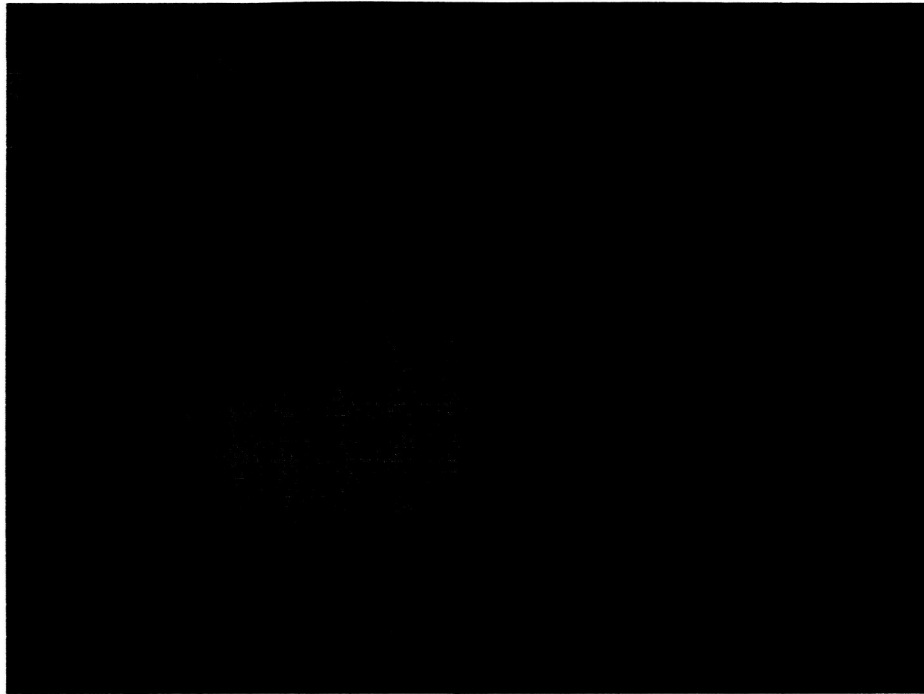
Bone surface feature is critical for wave propagation velocity, which is a primary component for bone's mechanical strength assessment. Confocal ultrasound scanning is able to detect surface topology of the material in high resolution (~50 micron).

SCAD – Accomplishment (4) - Automatic Irregular ROI



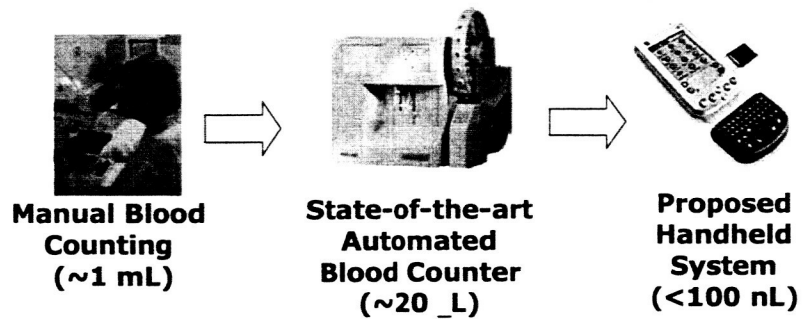
Next Steps

- Development of a practical/compact portable SCAD device for ground base use
- Development of SCAD hardware and software for multiple and critical skeletal sites, i.e., hip
- Analytical model for interpretation of SCAD data to bone quality
- Miniaturization
- Clinical application –
 - Osteoporosis assessment
 - Bedrest
 - Pre- / post flight astronauts



Project Goal:

"Automated Blood count" in Space



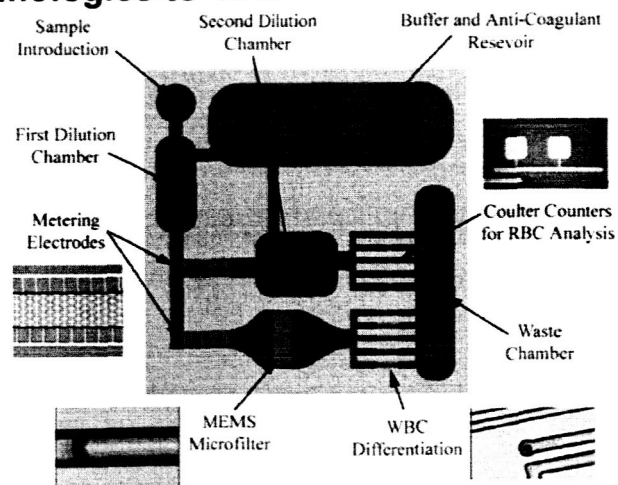
To develop a blood-count on-a-chip technology for space use.

Technical Goals

- Miniature Chip-based Cartridge
 - preloaded with reagents
 - disposable
- Analyzing a minimum of "1,000 RBC" and "200 WBCs",
 - processing sample volume ~50nL.
 - sample volume precisely measured $\pm 5\%$
 - On-chip sensing with built-in sensors.
- Blood count information
 - including RBC count, MCV, Hematocrit, WBC count and WBC (granulocyte) differential.

Technical Approach/Methodology:

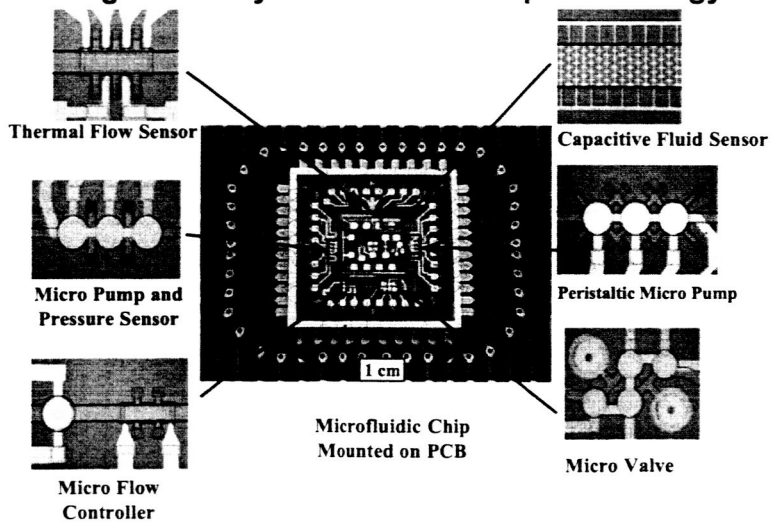
Combining MEMS and microfluidics technologies to build blood-count on-a-chip



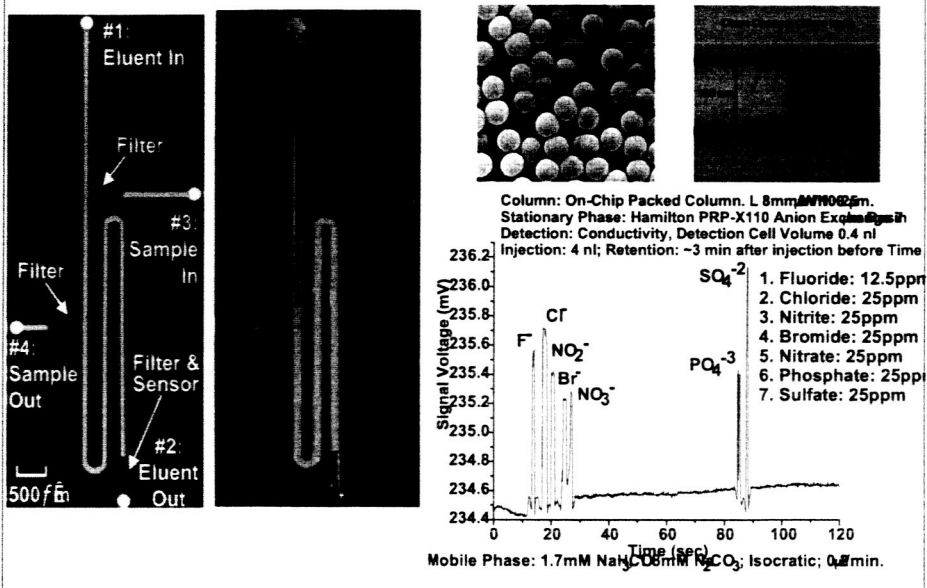
Blood Count Chip	Year 1	Year 2	Year 3	Year 4
1. RBC/WBC Separation				
RBC filter				
WBC filter				
2. Microfluidics (de-ionizer and cytometry)				
On-chip aspiration				
On-chip dilution				
3. Micro Coulter Counter				
RBC count				
RBC MCV (Coulter or capacitive sensing)				
Hematocrit (on-chip packed column)				
WBC count				
WBC differential (Coulter Counter or optical)				
4. System Integration in a box				
Instrument integration (shoebox form factor)				

Accomplishments

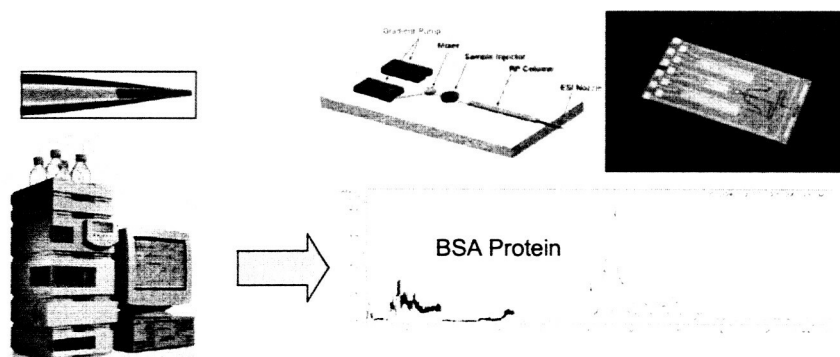
Integrated Parylene Lab-on-a-chip Technology



HPLC-on-a-Chip



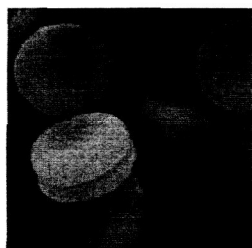
Gradient-Elution-HPLC-ESI On-a-chip



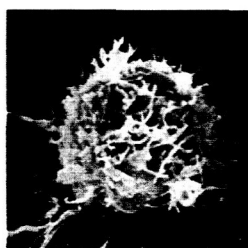
- The core function of a LC-ESI system is demonstrated
- The chip performance is comparable to current commercial system

Human RBC/WBC

	Cell Shape	Diameter Average (μm)	Diameter Range (μm)	Height (μm)	Count ($10^9/\text{L}$)
RBC	Biconcave disk		5 - 8	1.5 - 3.5	4,200 - 5,800
WBC	Sphere	10	7 - 20	7 - 20	4.5 - 11.0



RBC

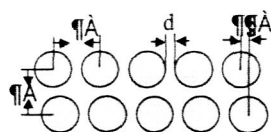


WBC (T Cell)



WBC engulf RBC

Cell Separation: Device Design and Fabrication



- Effective separation area 7mm x 1.8mm
- Upstream and downstream regions
- Column shift (—): 4 μm upstream, 6 μm downstream
- Center-to-center distance (—): 60 μm
- Distance (d): 14 μm

> Mold Lithography

> DRIE Channel

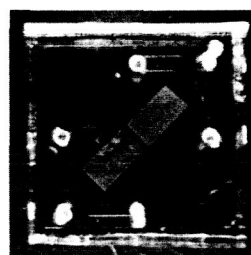
> PDMS

> Mold Channel

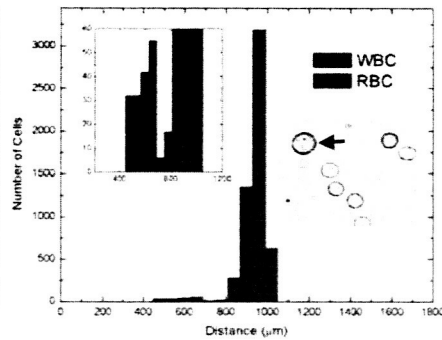
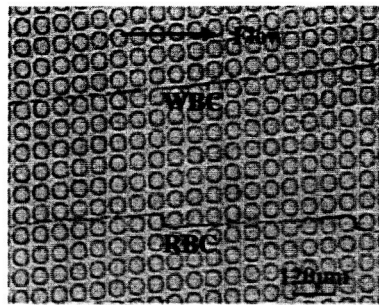
> Glass

> Punch Access Holes

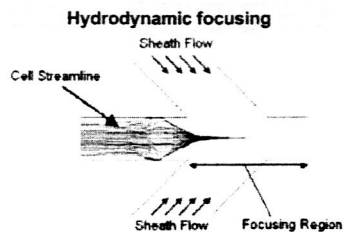
> Assemble to Glass Slide



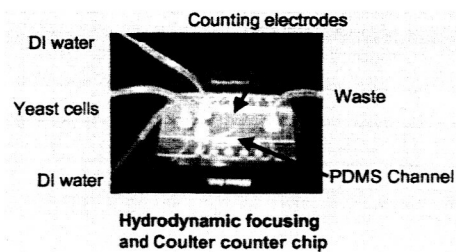
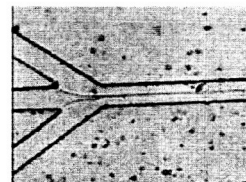
Separation of Human Blood Cells



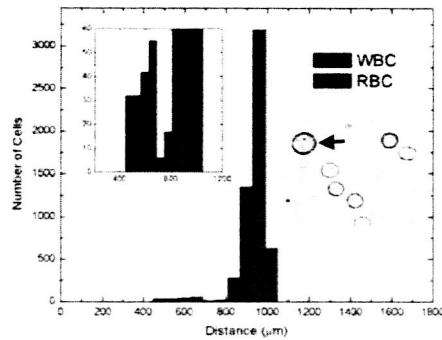
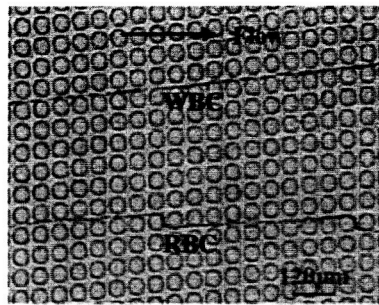
μ Cytometry with Hydrodynamic Focusing



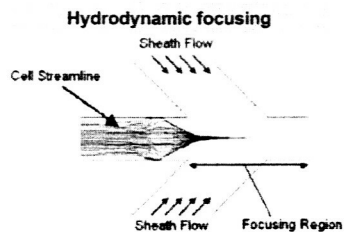
Hydrodynamic focusing of yeast cells



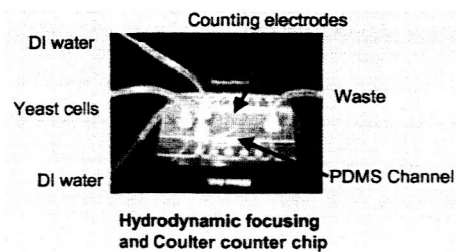
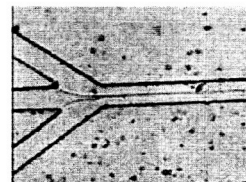
Separation of Human Blood Cells



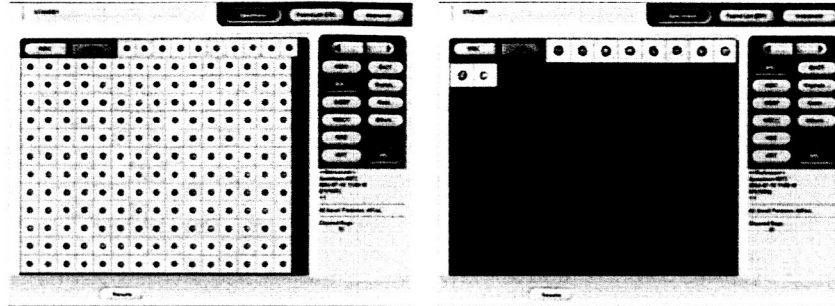
μCytometry with Hydrodynamic Focusing



Hydrodynamic focusing of yeast cells



Establishing Blood Count Reference



RBC & WBC Images from Iris Body Fluids
Instrument (1:1000 Dilution)

Next Steps

- Integration of various working components
- Design and fabricate sensors for blood cell
Size detection and differentiation
 - Coulter (impedance) sensor
 - Thermal sensor
 - Pressure sensor
- Design and fabricate RBC/WBC separation
device requiring minimal sample dilution
- Demonstrate separated RBC/WBC counts

Appendix D

National Space Biomedical Research Institute

Investigator Retreat, Jan. 12-15, 2004

The NSBRI Investigator Retreat was held January 12-15, 2004, at Del Lago Conference Center in Montgomery, Texas, with more than 260 persons attending. The retreat involved the principal investigators from each NSBRI research and education project, NASA counterparts, JSC Space and Life Sciences Management and Flight Surgeons, Code U representation, and members of NSBRI's international partners, External Advisory Council and Industry Forum. The format included interactive demonstrations highlighting accomplishments, products and deliverables, as well as plenary presentations from research and education team leaders and poster presentations from each funded project. NSBRI and NASA participants also held a half-day meeting on the Bioastronautics Critical Path Road Map and an evening meeting on the Technology Road Map.

Highlights

- Twenty-one interactive demonstrations highlighting products and deliverables. Descriptions of participating projects follow.
- Dr. Bill Paloski made a presentation about the Artificial Gravity Project.
- Dr. Rupert Gerzer of German Aerospace Center (DLR) Institute of Aerospace Medicine gave a presentation on the DLR's facilities and research collaboration prospects.
- Presentations were made by Dr. Jeffrey Sutton, Director, NSBRI; Dr. Jeff Davis, Director, NASA JSC Space and Life Sciences; Dr. Guy Fogleman, Director, NASA OBPR Bioastronautics; and Dr. Howard Ross, NASA Associate Deputy Administrator for Science, OBPR. Dr. Bobby R. Alford, NSBRI Chairman of the Board, also welcomed the participants.
- Retreat attendees participated in a half-day meeting on the Bioastronautics Critical Path Roadmap (BCPR) with individual teams meeting in small groups to further refine information for the BCPR.
- Education and Public Outreach Team members held a workshop on Critical Questions and Code N Mission and Education Requirements which included a presentation by Bonnie McClain, NASA Chief of OBPR Educational Outreach.
- Retreat attendees participated in an evening planning meeting on the Bioastronautics Technology Roadmap.
- Dr. Simon Ostrach, Director of National Center for Microgravity Research provided a briefing.
- A Team Leader Meeting was held to discuss transition of Team Leaders and projects.
- Individual teams held on-site meetings.
- New collaborations were developed, such as Dr. Rajulu becoming a part of the Muscle Alterations and Atrophy Team, and there were opportunities for the exchange of information between representatives of operations and research.

Retreat Interactive Demonstration Summaries

Cardiovascular Effects of Simulated Microgravity in Man

The technology for measuring microvolt T-wave alternans was developed under NASA/NSBRI support. This technology has been successfully commercialized by Cambridge Heart, Inc., (Bedford, Massachusetts) under license from the Massachusetts Institute of Technology. It is in widespread clinical use to assess non-invasively an individual's risk of ventricular tachyarrhythmias and sudden cardiac death. It is being used in this project to determine whether simulated microgravity alters cardiac electrical processes in a manner that may make the heart more susceptible to ventricular tachyarrhythmias.

Mechanisms of Cardiovascular Deconditioning

The device demonstrated was designed to maintain a person's normal arterial blood pressure in response to either an orthostatic challenge or from a hemorrhage. The device maintains the blood pressure by maintaining the cardiac output, or blood flow. This is done by augmenting the venous return, the blood flow returning to the heart, which is known to decrease during orthostatic challenges and hemorrhage. The method uses peristaltic compression, sequentially, from the lower limbs to the diaphragm. This peristaltic motion pushes the blood from lower extremities and abdomen to the right atrium of the heart which in turn augments the cardiac output and arterial blood pressure.

Diagnostic 3-D Echocardiography: Development of Novel Compression, Segmentation and Registration Techniques For Manned Space Flight Application

This demonstration illustrated three things: the transmission of wireless data from a portable ultrasound system to a handheld device; three-dimensional echocardiographic/ultrasound viewer/analyzer; and three-dimensional echocardiographic data compression.

In Vivo Stress-Strain Dynamics in Human Muscle

A leg-suspension system was used to unload one leg of a subject for four weeks to simulate microgravity. Phase contrast cine MRI data were collected during ankle movements. The data allowed investigators to reconstruct and thus non-invasively observe internal movements of the calf muscles. A viewer was developed for the Visible Human dataset in order to investigate the 3-dimensional structure of the calf muscles and help demonstrate the close relationship between the 3-dimensional structure of muscle and the motion of muscle tissues.

Countermeasures to Sleep and Circadian Disruption for Space Missions

The demonstration involved an overview of the Human Performance Factors, Sleep and Chronobiology Team's work, along with interactive demonstrations from two of the team's interrelated projects: 1.) A live-time demonstration of the Circadian Performance Simulation Software (CPSS), a computer program developed to assist in predicting the impact of work, sleep and lighting schedules on the alertness and performance of crew members. It has already been employed by NASA as a scheduling tool for recent space flight missions. 2.) A working demonstration of special prototype lamps developed specifically for research in optimizing the light spectrum as a countermeasure to sleep and circadian disruption. These lamps are predicted to be two-to-four times more effective than those currently used as a pre-launch countermeasure by NASA astronauts.

Inspiring the Next Generation of Space Life Scientists

Hands-on demonstrations of materials and curriculum developed by members of the NSBRI Educations and Outreach Team. The team's ongoing efforts are establishing NSBRI as a leading resource for bringing the excitement and importance of NSBRI space life sciences research into the nation's classrooms and homes.

Neurovestibular Aspects of Artificial Gravity Created by Short-Radius Centrifugation

Artificial gravity produced by high-speed rotations on a short-radius centrifuge can be a unique and cost-effective countermeasure against the degenerative effects of long-duration space flights. Rather than alleviating the microgravity symptoms, such as demineralization of the bones, muscle atrophy, and cardiovascular de-conditioning, it attempts to remove their cause. However, at rotation rates that induce around an Earth-like force at the feet (20-30 rpm), head and body movements create disruptive sensory conflicts. Limb movements will be deflected, and head movements induce motion sickness and inappropriate eye movements. This demonstration shows how humans adapt to head movements in a rotating environment. Within a few sessions, motion sickness score, illusionary body tilts, eye movements and deflection of arm movements are significantly reduced.

Measures to Counter Gait Ataxia

The demonstration involved the first prototype of a completely wearable device that provides vibrotactile feedback of measured body tilt using a 6 degree of freedom instrumentation system. The system is mounted on the small of the subject's back.

Balderini Gait Disturbance Device

Balderini, a portable balance platform for controlled perturbation of gait and posture in astronauts, was specifically designed to be implemented in the Functional Mobility Test (FMT), developed at NASA Johnson Space Center by Dr. Jacob Bloomberg and his collaborators. The FMT evaluates an astronaut's ability to perform challenging locomotor maneuvers, similar to those encountered during typical daily activities and especially during an egress from a space vehicle. This is accomplished by providing the astronaut obstacles to step over, duck under, and side step while receiving inadequate proprioceptive sensory information due to high-density visco-elastic foam covering the course. The Balderini system, which is designed to fit within one of the foam sheets covering the obstacle course, provides the means to evaluate an astronaut's ability to respond to an unexpected postural perturbation experienced during a functional activity. The Balderini system is comprised of a custom-designed, low-profile linear motor assembly connected to a platform on linear bearings. The linear motor is controlled from a PC and has a movement range of 20 cm, a maximum acceleration of 1-G, and a peak velocity of 1 m/s. The platform is instrumented with sensors that detect foot contact and foot location. An array of magnetic reed switches are used in combination with a series of small permanent earth magnets placed on the subject's footwear for non-contact detection of which foot will first contact the platform. The system, termed "Auto Foot," can automatically identify which foot is entering the platform, where it is placed, and if a perturbation should be triggered immediately or at a certain variable delay. A test session can be set up and run automatically using this system. The linear motor assembly is attached to the floor with industrial strength Velcro. This provides for a simple and highly portable mounting solution for the system.

Which Way Is Up? Mechanisms and Countermeasures for 0-G Visual Disorientation

Shuttle, Mir and ISS crews experience occasional disorientation due to inversion and visual reorientation illusions – “the downs.” These frame-of-reference shifts can cause a loss of sense of direction and spatial memory problems, particularly when moving between modules with different visual verticals. Crews visiting Mir sometimes became lost. Some of these problems can be recreated and quantified using the virtual-reality techniques demonstrated here. Crews currently train for EVA using neutral buoyancy and virtual-reality techniques. Preflight training could provide a useful disorientation countermeasure.

Joint MIHCS/NSBRI Multi-Specialty Medical Operations Support Team for Development and Testing of a Space-Adapted Human Patient Simulator

The demonstration involved video and live action of high-fidelity human patient simulation. Investigators also discussed how the Human Patient Simulator is utilized in integration and validation of medical education and procedures for flight crews, biomedical engineers and flight surgeons.

Optical Computer Recognition of Behavioral Stress

This demonstration was relevant to questions in Risk 21 of the Critical Path Roadmap (human performance failure because of neurobehavioral dysfunction). This project reflects a three-year collaboration between two laboratories: one with expertise in the evaluation of behavioral and physiological responses to stressful performance conditions (D. Dinges, University of Pennsylvania), and the other with expertise in optical computer recognition (OCR) of human expressions (D. Metaxas, Rutgers University). The goals are to develop “machine vision” that can track and discriminate facial expressions induced by low versus high workload performance demands, and to determine the influence that facial edema from microgravity, as well as gender, age, ethnicity, and alexithymia have on algorithm discriminability. This demonstration showed how significant advances in programming and enhancement of the capabilities of the OCR algorithm permit robust three-dimensional tracking of facial expressions of astronauts in space flight (using relatively low-quality stock video). The project poster on display showed the results of laboratory experiments used to develop the algorithm and its discriminability to low versus high performance stressors.

Speech Monitoring Cognitive and Personality Alterations: Climbing Mount Everest – A Space Analog

The project investigators have demonstrated that cognitive dysfunction that can arise during deep space flight from exposure to cosmic rays – particularly the ability to change plans in response to changing circumstances – can be remotely and unobtrusively monitored by acoustic measures of a person’s speech. The low oxygen content of the thin air on Everest produces similar brain dysfunction with high stress levels arising from life-threatening danger, yielding an ethical “space analog.” This exhibit demonstrated the techniques that have been developed, the space and earthbound applications, and plans to develop monitoring techniques for other possible aspects of cognitive dysfunction and personality shifts arising from exposure to radiation and stress.

Quick Assessment of Basic Cognitive Function: “Blood Pressure Cuffs” for the Mind

MiniCog is a series of short, easy-to-administer tests likened to “blood pressure cuffs” for the mind to evaluate space travel’s effects on cognitive function. These tests, administered through a user’s Palm Pilot, deliver objective, immediate assessment of cognitive function. This program can also be used to assess the effects on cognitive function of various Earth-bound activities, including the effects of sleep loss, of physical exercise, of hormonal variations, or even of eating certain foods. This demonstration exhibited five Palm Tungstens with MiniCog installed for users to try the test battery that has been developed. Attendees were also able to see how tests are created and how data is transferred.

Near-Infrared Brain Imaging for Space Medicine

This demonstration illustrated:

- Brain Recording Demo: Attendees had the opportunity to see brain function being monitored by near-infrared spectroscopy (NIRS), with recordings viewable in real-time. Physiology waveforms were also be available for comparison to the NIRS data.
- SpaceDock Demo: Participants were able to test-drive the dual-joystick simulated docking task – SpaceDock – developed for and used in studies of sleep-deprivation and brain function.
- Automated Diagnosis Demo: Attendees saw a computational demonstration system that exhibits the feasibility of multi-sensor fusion and interpretation for automated medical diagnosis.

Guided High Intensity Focused Ultrasound for Mission-Critical Care

Internal bleeding is one of the most difficult medical conditions to treat, especially in the space environment where full medical facilities are not available. This project involves the design of a lightweight, portable device that would use diagnostic ultrasound to determine a site of bleeding and High Intensity Focused Ultrasound to induce hemostasis. The device could also be used for a number of other medical conditions, such as the identification and treatment of benign and malignant tumors. This demonstration showed a guided high intensity focused ultrasound device to detect and to arrest bleeding.

Minimally Invasive Diagnosis and Therapy of Microgravity Medical Contingencies/Advanced Ultrasound Diagnosis in Microgravity

Several crew health situations, such as severe abdominal pain, dental or sinus infection, musculoskeletal injury, and eye trauma, could severely impact the success of long-duration missions. Diagnosing and managing acute health problems on these flights is problematic due to several factors, including lack of information on how microgravity impacts anatomy, disease presentation and therapy effectiveness. This team is studying the ability to use miniature laparoscopy, in combination with ultrasound, in patient studies involving select health situations which would have a high impact on mission success. These diagnostic tools will be evaluated in animal models under simulated microgravity situations. Information gained will be used to optimize training regimens and refresher modules for non-physician crew medical officers to diagnose and treat astronaut medical conditions during long missions. The diagnostic, treatment and training protocols which will be developed in this study will also provide information for use in rural care, military conflicts and third world medicine. The demonstration highlighted a CD-

ROM based remote guidance instructive module for use in remote environments to train non-physicians advanced medical techniques.

Noninvasive Measurement of Blood and Tissue Chemistry

The demonstration involved a noninvasive metabolic monitor based on near infrared spectroscopy for the assessment of blood hematocrit, regional muscle pH and PO₂ measurement. Novel components include a highly stable light source; optical bench, control software and fiber optic cable for real-time referencing; and innovative target for precise placement of fiber probe on the hand. The compact system acquires a spectrum which is processed with calibration equations, developed as part of this project, to quantitate all three analytes from a single spectrum. The system is general purpose and could be used for other optical spectroscopic applications.

Health Consequences of Radiation in Space

The demonstration outlined the NSBRI Radiation Effects Team's contribution to ground-based studies using NASA facilities at the Brookhaven National Laboratory.

Improved Bubble Detection for EVA

Astronauts who perform spacewalks are at risk for decompression sickness – “the bends.” If decompression sickness occurs, it can cause severe joint pain, coughing, skin irritation, cramps and even paralysis due to nitrogen bubbles in the blood and tissue. This project is testing ultrasonic instruments that detect small nitrogen bubbles in both blood and tissue to monitor for and prevent decompression sickness. If successful, these instruments will lead to better understanding and detection of decompression sickness in astronauts as well as deep-sea divers and pilots. This demonstration included a video description of the design and function of the bubble detection and sizing unit.

Real-Time Analysis of Biomarkers and Countermeasures Using a Miniature Time-of-Flight Mass Spectrometer

A miniature mass spectrometer to monitor human physiological functions routinely and non-invasively during flight was demonstrated by video. The device analyzes urine, blood and breath to detect all levels of chemical and biological substances with low error rates. This small, lightweight, low-power machine will provide information about physical health, muscle atrophy, bone loss and sleep regulation.

Appendix E



SUMMARY OF INSTITUTE ACTIVITIES

Board of Directors Meeting

March 18, 2004

NSBRI continues to lead robust research and education programs which are well aligned with its mission and NASA's guiding principles for space exploration. Earlier this year, the Institute delivered its first two countermeasure products. It implemented an effective process of tracking deliverables, created new mechanisms to fill gaps in the research and development pipeline, especially in the countermeasure readiness level (CRL) 6-9 range, and became more engaged in flight activities (e.g., on-board cardiac ultrasound on ISS increments 7 and 8). The appointment of Jeanne Becker, Ph.D., as Associate Director, has been well received by the NSBRI and NASA communities, and her role as the Institute's chief scientist is serving to further strengthen leadership in the NSBRI/NASA partnership.

The Institute hosted a successful biennial investigator retreat in January 2004. Among the highlights were hands-on demonstrations of more than twenty research countermeasures in development and a series of team leader lectures summarizing the breadth and depth of NSBRI accomplishments. There were also strategic planning sessions for the Critical Path Roadmap, technology roadmap and integrated education and outreach programs.

It was fortuitous that during the retreat, President Bush articulated a renewed spirit of discovery for NASA and the nation through a robust human and robotic space exploration program. This program, which is now NASA's highest priority, provides NSBRI with unique and increased opportunities to scientifically and technologically contribute to biomedical risk mitigation for long-duration human space travel. NSBRI has been in deliberations with NASA regarding expanded roles and budget within Bioastronautics, given the Institute's measurable productivity, integrated expertise and resources, accumulated experience, scalability and alignment with the President's vision for U.S. space exploration.

For extended-duration human space missions, accelerated bone loss remains a high risk. Jay Shapiro, M.D., a professor at USUHS and former team leader of the NSBRI Bone Loss Team, has completed a six-year project demonstrating the usefulness of a single, 15-minute infusion of a bisphosphonate, zoledronate, in significantly reducing the extent of bone loss for up to one year after injection. This finding, resulting from a research study in spinal cord injury patients in an analog bedrest setting, has pushed zoledronate to the countermeasure evaluation and validation project (CEVP) phase of development. This is new CRL territory (CRL 7-8) for NSBRI, and the Institute is working in partnership with NASA to jointly assess zoledronate for flight.

Richard Potember, Ph.D., an investigator at The Johns Hopkins University Applied Physics Laboratory and member of the NSBRI Technology Development Team, has completed six years of work on a time-of-flight mass spectrometer, which has broad applications for in-flight science experimentation and medical assessment. This is timely given the limited up and down mass of ISS samples, and the need to expand on-board capabilities for exploration missions. NASA's Fundamental Space Biology program, as a potential customer, has expressed interest in seeing Dr. Potember's small, portable, lightweight and low-power device reach flight status.

The objectives to transition the research portfolio to higher CRLs, to sustain continuity of support of highly meritorious projects and programs, and to openly compete research proposals and leadership positions, have been of paramount importance for the Institute. NSBRI received 111 proposals for team-based countermeasure research. Additionally, NASA received 108 proposals for individual countermeasure projects and the NSBRI Board of Scientific Councilors served on peer review panels for both NSBRI and NASA. Forty-three NASA proposals received a merit score of 70 or greater, whereas 63 NSBRI proposals fell in this range, reflecting the high caliber of investigators applying to the Institute.

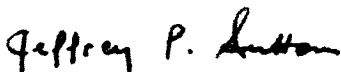
Meritorious proposals received by NASA, with high CRLs and relevance to NSBRI teams, were considered for NSBRI support, while low CRL proposals received by NSBRI were sent to NASA for funding consideration. The External Advisory Council (EAC) met twice to discuss research proposals, including programmatic relevance as assessed by strategic need, product development and return on investment. NSBRI subsequently selected 47 projects, with FY04 and FY05 core obligations of \$6.0 M and \$15.8 M, respectively. NASA agreed to support two of these projects over and above the current \$30 M core support to the Institute. The majority of selected projects were competitive renewals, with the result that the research program will have 64 projects, funded at a higher average level of support and on more tightly focused teams, at the beginning of FY05 relative to 92 projects at the beginning of FY04. Approximately \$2.8 M of core funds will be available in FY05 for selection of additional projects, resulting in an estimated 70 projects supported at the beginning of FY06. NSBRI is currently working on a targeted research announcement with multiple opportunities, including solicitation of program projects, for release in the late spring 2004.

NSBRI received 21 applications in response to its open announcement for team leadership positions. Following vetting through the EAC, six team leaders were successful in being re-appointed, two associate team leaders were elevated to team leader status, and one investigator, formerly not funded by NSBRI, was appointed as a team leader. Team leaders had an average peer review merit score of 88 (top 7% of investigators). Thus, the Institute continues to have outstanding scientists with excellent leadership capabilities head its research teams.

Jonathan Clark, M.D., NSBRI/NASA Space Medicine Liaison, and Edna Fiedler, Ph.D., NSBRI/NASA Behavioral Health Liaison, have made significant contributions in bridging NSBRI team leader/research efforts with operational personnel and customers. The Institute has also broadened its presence in NASA programs through the NSBRI/NASA JSC Steering Committee, Bioastronautics Science Management Team, Bioastronautics tag-ups, Space Medicine Lead Docs meetings, NASA's Radiation Board and a newly created NSBRI/CEVP working group

The Institute continues to excel in education and outreach efforts, and has received high praise from within NASA and the external community. A post-doctoral program is ready for launching in April, the 2004 summer internship program is quickly filling up and a request for proposals for K-16 and graduate training projects will be released in the spring 2004.

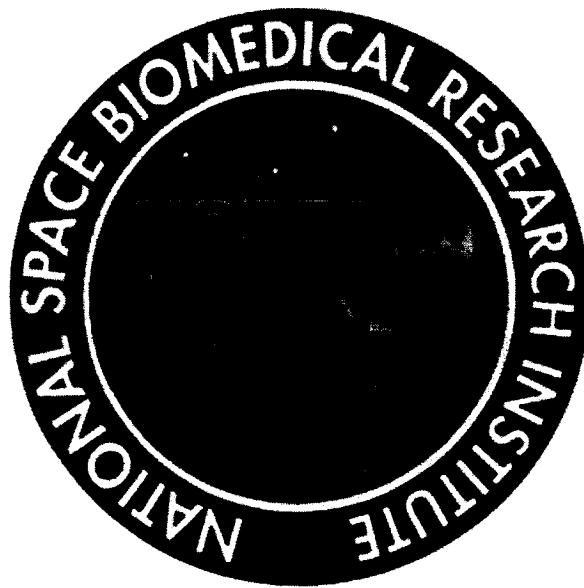
Respectfully submitted,



Jeffrey P. Sutton, M.D., Ph.D.
Director

Appendix F

**NATIONAL
SPACE BIOMEDICAL
RESEARCH INSTITUTE**



**Core Research Program
Publications and Presentations List**

October 1, 2003 – September 30, 2004

National Space Biomedical Research Institute Publications

Bone Loss Team

Articles

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Rivera, C. A., M. H. Tchamitchi, L. Mendoza, and C. W. Smith. Endotoxemia and hepatic injury in a rodent model of hindlimb unloading. *J Appl Physiol* 95(4):1656-1663, 2003.

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Judex, S., N. Zhong, M. Squire, R. Garman, L. R. Donahue, M. Hadjiargyrou, and C. T. Rubin. Mechanical modulation of molecular signals which regulate anabolic and catabolic activity in bone tissue. *Journal of Cellular Biochemistry*, in press.

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National Space Biomedical Research Institute Publications

Neurobehavioral and Psychosocial Factors Team

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National Space Biomedical Research Institute Publications

Nutrition, Physical Fitness and Rehabilitation Team

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National Space Biomedical Research Institute Publications

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National Space Biomedical Research Institute Publications

Smart Medical Systems Team

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National Space Biomedical Research Institute Publications Technology Development Team

Presentations

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Davis, B. L., E. Eucker, and S. E. D'Andrea. Design and development of an exercise countermeasure device for possible use during long-duration space missions. XIIIth International Conference on Mechanics in Medicine and Biology, Tainan, Taiwan, November 12-15, 2003.

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National Space Biomedical Research Institute Publications Education and Public Outreach Team

Articles

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Appendix G

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

POLICY ON TEAM LEADERSHIP

I. Overview

Each Institute team is led by a single Team Leader who is assisted by an Associate Team Leader. Team Leaders play a pivotal role in guiding the Institute's research program and the ultimate success of the Institute. Their expertise and "hands-on" approach to research management add value across projects and across teams. The Team Leader is guided by the Critical Path Roadmap (CPR), which is the cornerstone for developing the team's integrated strategic research plan, the key to accomplishing the Institute's mission. The Team Leader's stature and reputation as a strong scientist encourages other scientists to apply to become team members. The Team Leader's communication skills and insight enable the appropriate synergistic discussions among the various research projects, with the objective of assuring a team research program that has higher value than the sum of the values of its separate projects.

II. Duties and Responsibilities

Team Leaders are responsible for:

- Preparing and periodically updating the team research strategic plan. This plan should be consistent with the Institute mission, the CPR, and available resources.
- Reporting progress to the Institute's External Advisory Council (EAC), Board of Scientific Counselors (BSC), and NSBRI management.
- Preparing and presenting the initial recommendation to the EAC of new research projects for inclusion in the team's program.
- Representing the team and disseminating knowledge about team activities and progress to NASA; specifically coordinating with NASA-JSC scientists and physicians, the scientific community, and the general public.
- Pursuing involvement with NASA operational activities.
- Maintaining appropriate communication links among the team investigators and to other team leaders.
- Developing, with team investigators, individual project plans that ensure scientific and operational synergy and lead to productive countermeasure development.
- Nurturing opportunities and seeking funding support to collaborate with, and cross-fertilize, research within and between NSBRI teams and with Johnson Space Center, other NASA Centers, and other agencies.
- Acting as the senior NSBRI discipline representative for ongoing development of the CPR.

Associate Team Leaders assist Team Leaders in carrying out the above activities.

III. Qualifications

Team Leaders are NSBRI-funded principal investigators who possess the following qualifications:

- Achieved intermediate or senior rank at a research or educational institution.
- Demonstrated record of securing independent competitive research funding for the last five years, at least.
- Recognized within the biomedical community as an outstanding research contributor to at least one field of study; prior involvement with a NASA flight investigation would be beneficial.
- Manifest broad scientific understanding across the team's research area.
- Demonstrated leadership and program/group management skills, as evidenced by experiences such as a section head, department chair, dean, research center director, or principal investigator on a program project.
- Exhibit good communication, public speaking, and organizational skills.
- Show a willingness and availability to spend the necessary time and energy to fulfill the role of Team Leader.

Associate Team Leaders are principal or co-investigators on NSBRI-funded projects who possess at least the first four of the above qualifications required for a Team Leader. Generally, Team Leaders and Associate Team Leaders are not from the same institution.

IV. Term of Service

Team Leaders are appointed by the Director for a term that is identical with the term of their NSBRI-funded research project (generally four years), subject to satisfactory performance as determined at their annual performance review. The Team Leader appoints an Associate Team Leader for a term that does not exceed the Team Leader's term of service. The Team Leader's term is competitively renewable.

V. Funding and Authority

Team Leaders and Associate Team Leaders are provided with discretionary funds to enable them to carry out their duties and responsibilities. Wide latitude is provided concerning the expenditure of these funds within the guidelines of the involved institutions. Such funds may be used for support personnel, team meetings, special travel, and other expenses generally associated with team communication and operations. However, these funds may not be used to support research.

Team Leaders are ultimately responsible for carrying out the duties and responsibilities listed in Section II. They are expected to work cooperatively with their Associate Team Leader in all matters and should develop a clear understanding of the distribution of their shared responsibilities. Team Leaders report to the Director.

VI. Selection

In the year before a Team Leader's term of service ends, a special "Call for Candidates" Announcement requesting applications for the Team Leader's position will be released in coordination with the annual Institute Research Announcement. Following the

evaluation of the research application by a peer committee, Institute Senior Management (Director and Associate Director) will evaluate, with EAC input, the merits of the applicants for Team Leader. The Director will recommend a selection to the Chairman of the Board who will seek confirmation of the selection from the Board of Directors.

Associate Team Leaders are nominated and selected by the Team Leader, with the advice and consent of the Director in consultation with the Associate Director.

In selection of Team Leaders and Associate Team Leaders, attempts will be made to balance the scientific and managerial expertise of candidates and to develop diversity within the Institute's research leadership.

VII. Training and Support

To assist Team Leaders in performing their duties, the NSBRI provides electronic reporting and managerial tools, along with training as needed. Forums are held at least three times a year for Team Leaders to meet as a group with the Director and Associate Director.

VIII. Performance Evaluation

Once a Team Leader is selected, five groups evaluate the performance and effectiveness of Team Leaders: the EAC, BSC, team principal investigators, NSBRI Senior Management, and NASA. Each group focuses on different aspects of a Team Leader's performance:

- Annually, the BSC will review each team's annual report of productivity and progress in carrying out the team strategy, including evidence that the research projects are functioning synergistically within the research team and evidence that the team is collaborating effectively with other teams and with NASA life scientists.
- Semi-annually, the EAC will review the effectiveness of the Team Leader in communicating the team vision and successes, and in discussing and handling team issues and problems.
- Annually, team principal investigators will evaluate the leadership, communication, and other relevant skills of their Team Leader.
- Annually, Institute Senior Management will evaluate the Team Leader's overall effectiveness and responsiveness.
- At least every four years, and more frequently if necessary, the four research area representatives on the EAC and BSC (two each) will review the team strategic plan and furnish a written critique of the strengths and weaknesses of the plan along with a rating of the overall team strategy embedded in the plan.
- Every five years, just prior to conducting an Institute-wide review, an *ad hoc* review team, appointed by NASA, will evaluate all aspects of the team's performance, including the Team Leader's performance.

Institute Senior Management will produce an annual overall rating of each Team Leader's performance based on the available inputs.

An unsatisfactory Team Leader rating will normally result in a specific warning to the Team Leader and include a recommended action plan to correct the identified deficiencies in performance. Two unsatisfactory Team Leader ratings in successive years will result in removal of the Team Leader and appointment of an acting Team Leader to serve out the remainder of the Team Leader's term. The Institute supports the need for leadership continuity but only if the evaluative process supports an annual reappointment. Team Leaders are ultimately judged by their team's ability to successfully develop and deliver, in whole or part, countermeasures in areas of high impact for NASA, for the purpose of decreasing the biomedical or human performance risks associated with long-duration human space flight.

Associate Team Leaders are evaluated annually by Team Leaders for their contribution to team goals, achievements, function, productivity, and representation. Unsatisfactory performance may lead to removal of Associate Team Leaders, but such action requires the concurrence of the Director in consultation with the Associate Director.

IX. Conflict of Interest

Team and Associate Team Leaders must adhere to the highest ethical standards as they carry out their leadership duties. They must not make decisions based on institutional affiliation or personal bias. They must conduct all leadership duties with integrity, fairness, and objectivity to ensure the scientific credibility of the Institute.

To avoid a conflict of interest during a selection in which a Team Leader has a competing application, Institute Senior Management selects the Team Leader and project before any other projects are selected. Then the Team Leader develops and presents a selection recommendation concerning the other competing projects to the EAC. The EAC recommends the final selection to Institute Senior Management, taking into account the science merit rating and programmatic relevance rating furnished by the BSC in addition to the Team Leader recommendation. Institute Senior Management makes the final selection decisions following coordination with NASA. If the Team Leader does not have a competing application during a selection cycle, the process is similar but the Team Leader will have input in developing the selection recommendations to the EAC.

Appendix H

Note: Document Altered to Remove Merit Scores

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

**SELECTION MEETING
NRA 03-OBPR-04 PROPOSALS
SUBMITTED BY TEAM LEADER CANDIDATES**

December 16, 2003

Attendees

NSBRI

Jeffrey Sutton, M.D., Ph.D., Chair
Bobby Alford, M.D.
Jeanne Becker, Ph.D.
Edna Fiedler, Ph.D.
Kathryn Bruning
James Cooper
Kathy Major
Paul Lampi
Mary Lee

NASA Headquarters (by telecom)

Guy Fogleman, Ph.D.
David Tomko, Ph.D.
Bruce Hather, Ph.D.

NASA JSC

Charles Sawin, Ph.D.

Background

On April 15, 2003, NRA 03-OBPR-04 was released soliciting ground-based research proposals for the Biomedical Research and Countermeasures (BR&C) Program. NSBRI received 111 proposals in response to the call to submit research proposals for one of ten NSBRI research teams. All proposals were peer-reviewed.

The Institute also released, on April 15, 2003, an open call for candidates, NSBRI CFC-03-01, soliciting applications for team leadership. Twenty-one submissions were received. NSBRI CFC-03-01 and NRA 03-OBPR-04 were independent announcements and no cross-referencing of applications was permitted in the proposals.

Following peer-review and assignment of merit scores, NRA 03-OBPR-04 proposals, which were submitted by investigators who also responded to NSBRI CFC-03-01, were identified. Fourteen of these proposals had merit scores of 70 or greater, and were subsequently assessed for programmatic relevance and cost. Relevance assessment was conducted by members of the NSBRI Executive Science and Medicine Council and NASA, including Drs. Jonathan Clark and John Charles. The assessment of cost included the NSBRI manager of finance.

Research proposals, peer-review comments and merit scores, relevance and cost assessments, and team leadership applications were then brought to the NSBRI External Advisory Council (EAC) for discussion. An executive session of the EAC was held on December 10, 2003, and NASA was present. The discussion focused on leadership attributes, proposed research and impact of selection recommendations for the Institute and the Bioastronautics program. Ten research proposals were recommended for selection (see attached spreadsheet with scores).

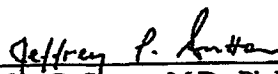
Selection

The following research proposals were discussed and selected by NSBRI, with NASA concurrence:

PI	Organization	Proposal Title
Cavanagh, Peter R.	The Cleveland Clinic Foundation	Foot Reaction Forces During Simulated ISS Exercise Countermeasures
Shoukas, Artin A.	The Johns Hopkins School of Medicine	Non-Adrenergic Mechanisms of Cardiovascular Deconditioning
Czeisler, Charles A.	Brigham & Women's Hospital	Circadian Entrainment, Sleep-Wake Regulation and Neurobehavioral Performance During Extended Duration Space Flight
Kennedy, Ann R.	University of Pennsylvania School of Medicine	Countermeasures for Space Radiation Induced Myeloid Leukemia
Baldwin, Kenneth	University of California Irvine	Force Regulation As a Countermeasure To Muscle Atrophy
Dinges, David F.	University of Pennsylvania School of Medicine	Optical Computer Recognition of Performance Under Stress
Oman, Charles	MIT Man Vehicle Laboratory	Visual Orientation, Navigation, and Spatial Memory Countermeasures
Lupton, Joanne R.	Texas A&M University	Nutritional Countermeasures to Radiation-Enhanced Colon Cancer
Crum, Lawrence A.	University of Washington	Smart Therapeutic Ultrasound Device for Mission-Critical Medical Care
Buckey, Jay C.	Dartmouth Medical School	Improved Bubble Detection for EVA

Action Items

1. Dr. Jay Buckey will be asked to coordinate his research with Dr. Mike Gernhardt's work at NASA JSC.
2. NSBRI and NASA will work together to ensure that funding of Dr. Crum's project takes into proper consideration the participation of Russian scientists.
3. NSBRI will resolve any time over-commitment issues of investigators prior to funding.
4. NSBRI will provide a briefing to NASA HQ on the relevance scale developed for translational research.
5. NSBRI will inform the NASA Radiation Board of selected projects led by Drs. Kennedy and Lupton.
6. NSBRI and NASA will continue to work together to coordinate selection for the remainder of BR&C proposals from NRA 03-OBPR-04.


 Jeffrey P. Sutton, M.D., Ph.D.
 Director, NSBRI

12/17/03
 Date

Encl.

NSBRI Research Proposals (Submitted by Candidates Seeking Team Leadership) and Merit Scores
Sorted by Team

CONFIDENTIAL

Team Name	Proposal #	Applicant Name	Applicant Organization	Proposal Title	Merit Score	Ordinal Rank (Total)*	Percent Rank*	Adjusted Relevance Score	Category
Bone Loss	0097	Cavanagh, Peter R.	The Cleveland Clinic Foundation	Foot Reaction Forces During Simulated ISS Exercise Countermeasures					
Cardiovascular Alterations	0041	Shaykue, Arlin A.	The Johns Hopkins School of Medicine	Non-Adrenergic Mechanisms of Cardiovascular Deconditioning					
Human Perf. Fac., Sleep & Chronobiology	0069	Czeisler, Charles A.	Bridham & Women's Hospital	Circadian Entrainment, Sleep-Wake Regulation and Neurobehavioral Performance During Extended Duration Space Flight					
Immunology, Infection & Hematology	0098	Kennedy, Ann R.	University of Pennsylvania School of Medicine	Countermeasures for Space Radiation Induced Myeloid Leukemia					
Muscle Alterations	0011	Baldwin, Kenneth	University of California Irvine	Force Regulation As a Countermeasure To Muscle Atrophy					
Neurobehavioral and Psychosocial Factors	0031	Dinges, David F.	University of Pennsylvania School of Medicine	Optical Computer Recognition of Performance Under Stress					
Neurovestibular Adaptation	0044	Oman, Charles	MIT Man Vehicle Laboratory	Visual Orientation, Navigation, and Spatial Memory Countermeasures					
Nutrition, Physical Fitness and Rehabilitation	0049	Lupton, Joanne R.	Texas A&M University	Nutritional Countermeasures to Radiation-Enhanced Colon Cancer					
Smart Medical Systems	0013	Crum, Lawrence A.	University of Washington	Smart Therapeutic Ultrasound Device for Mission-Critical Medical Care					
Technology Development	0022	Buckley, Jay C.	Dartmouth Medical School	Improved Bubble Detection for EVA					

*Ordinal and percent ranks within peer review panels in which NSBRI and NASA proposals were evaluated

SUMMARY OF TEAM LEADER RESEARCH PROPOSALS

Merit Score Range	60-95
Average Merit Score	87.6
Average Percent Rank	93.3%
Proposal Category	I 8
	II 2

Appendix I

Note: Document Altered to Remove Merit Scores

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

SUMMARY OF SELECTION MEETINGS FOR NRA 03-OBPR-04 PROPOSALS

May 10, 2004

Attendees at March 4, 2004 Meeting, NPRS, Washington

NSBRI

Jeffrey Sutton, M.D., Ph.D.
Jeanne Becker, Ph.D.
Kathryn Bruning
Paul Lampi

NASA Headquarters

Guy Fogleman, Ph.D.
Gale Allen, Ph.D.
David Tomko, Ph.D.
Victor Schneider, M.D.
Bruce Hather, Ph.D.
Bette Siegel, Ph.D.

NASA JSC

Charles Sawin, Ph.D.

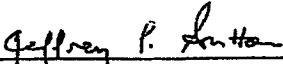
InDyne, Inc
David Watson, Ph.D.
Charles Liarakos, Ph.D.

On April 15, 2003, NRA 03-OBPR-04 was released soliciting ground-based research proposals for the Biomedical Research and Countermeasures Program. NSBRI received 111 proposals and NASA received 108 proposals. Following peer review for merit, ten of the NSBRI proposals were selected by NSBRI, with NASA present, on December 16, 2003. A document, entitled "NSBRI Selection Meeting NRA 03-OBPR-04 Proposals Submitted by Team Leader Candidates" provides a record of the selections.

The remaining 101 peer-reviewed NSBRI proposals were subsequently considered for funding. Additionally, 14 proposals submitted to NSBRI and 11 proposals submitted to NASA were considered for funding by NASA and NSBRI, respectively. NSBRI assessed proposals for relevance and cost, as described in the NRA, and categorized, based on a merit-relevance/cost matrix, into levels I (highest) through IV (lowest) [see attached]. For the 101 NSBRI proposals, there were 14 category I, 30 category II, 15 category III and 42 category IV proposals. For the NASA proposals under consideration by NSBRI, two were category I, 8 were category II and one was a category IV proposal.

The proposals were presented to the NSBRI External Advisory Council on February 10-12, 2004 for discussion and prioritization within and across research teams. On March 3, 2004, NSBRI met with NASA HQ and JSC personnel in Washington to commence selection. With the exception of a decision on one technology proposal relevant to radiation, the selection process was completed in a follow-up telecon between NSBRI and NASA on March 16, 2004. The NSBRI Director brought the remaining radiation technology proposal before the NASA Radiation Board, and the project was subsequently selected by NSBRI on May 10, 2004.

The merit scores and categorization of the 38 selected proposals are included as an appendix.


Jeffrey P. Sutton, M.D., Ph.D.
Director, NSBRI

5/12/04
Date

Encl.

**Non-Team Leader Selected Proposals
National Space Biomedical Research Institute
NRA 03-OBPR-04 Proposals**

Team: Bone Loss

PI Name	PI Organization	Proposal Title	Merit Score	ARS	Cat.
Bloomfield, Susan	Texas A&M Research Foundation	Increasing the Efficiency of Exercise Countermeasures for Bone Loss			
Bateman, Ted	Clemson University	Examination of Anti-Resorptive and Anabolic Treatments/Stimuli on Unloading Induced Osteoporosis			
Smith, Carolyn	Baylor College of Medicine	Receptor Countermeasures to Bone Loss in Microgravity			
Midura, Ronald	The Cleveland Clinic Foundation	Effects of Simulated Weightlessness on the Repair of Lower Limb Bone Fractures and on the Number of Bone-derived Stem Cells			
Schaffler, Mitchell	Mount Sinai School of Medicine	Bone Recovery Potential After Bisphosphonate and PTH Treatment of Disuse Osteoporosis			

Team: Cardiovascular Alterations

PI Name	PI Organization	Proposal Title	Merit Score	ARS	Cat.
Williams, Gordon	Brigham and Women's Hospital	Effects of Microgravity on Renal, Endocrine and Volume Regulatory Function			
Cohen, Richard	Massachusetts Institute of Technology	Effects of Simulated Microgravity on Cardiovascular Stability			
Mark, Roger	Massachusetts Institute of Technology	Computational Models of Cardiovascular Function for Simulation, Data Integration, and Clinical Decision Support			
Ray, Chester	Pennsylvania State University	Ultrasonic Bone Stimulation: Countermeasure to Orthostatic Intolerance			

Team: Human Performance Factors

PI Name	PI Organization	Proposal Title	Merit Score	ARS	Cat.
Dinges, David	University of Pennsylvania	Countermeasures to neurobehavioral deficits from cumulative sleep deprivation during space flight: Dose-response effects of recovery sleep opportunities			
Brainard, George	Thomas Jefferson University	Optimizing Light Spectrum for Long Duration Space Flight			
Klerman, Elizabeth	Brigham and Women's Hospital	Mathematical Modeling of Circadian/Performance Countermeasures 2004			
Menaker, Michael	University of Virginia	A Model of Circadian Disruption in the Space Environment			
Tosini, Gianluca	Morehouse School of Medicine	Preventing Desynchronization of the Circadian System in Long-Term Space Flight			

Team: Immunology, Infection & Hematology

PI Name	PI Organization	Proposal Title	Merit Score	ARS	Cat.
Gewirtz, Alan	University of Pennsylvania	Effect of Deep Space Radiation on Human Hematopoietic Stem and Progenitor Cell Function			
Shi, Yufang	Robert Wood Johnson Medical School	Apoptosis and Immune Homeostasis During Hindlimb Unloading			
Butel, Janet	Baylor College of Medicine	Biology of Virus Infections: Radiation and Immunity			
Sonnenfeld, Gerald	Morehouse School of Medicine	Bed Rest and Immunity (Funded with Non-Core Dollars to NSBRI)			

Team: Muscle Alterations & Atrophy

PI Name	PI Organization	Proposal Title	Merit Score	ARS	Cat.
Caiozzo, Vincent	University of California, Irvine	Hypergravity Resistance Training: Countermeasure to Microgravity			
Goldberg, Alfred	Harvard University Medical School	The Activation Of Protein Breakdown In Muscle Upon Unloading And Possible Countermeasures			

Team: Neurobehavioral and Psychosocial Factors

PI Name	PI Organization	Proposal Title	Merit Score	ARS	Cat.
Brady, Joseph	The Johns Hopkins School of Medicine	Psychosocial Performance Factors in Space Dwelling Groups			
Carter, James	Beth Israel Deaconess M.C.	Self-guided Depression Treatment on Long-duration Space Flights: A Continuation Study			
Orasanu, Judith	NASA Ames Research Center	Enhancing Team Performance for Exploration Missions			
Kosslyn, Stephen	Harvard University	MiniCog: A Portable and Fast Assessment of Cognitive Functions			
Lieberman, Philip	Brown University	Speech Monitoring of Cognitive Deficits and Stress			

Team: Neurovestibular Adaptation

PI Name	PI Organization	Proposal Title	Merit Score	ARS	Cat.
Bloomberg, Jacob	NASA Johnson Space Center	Development of a Gait Adaptability Training Program as a Countermeasure for Postflight Locomotor Dysfunction			
Young, Laurence	Massachusetts Institute of Technology	Neurovestibular aspects of short-radius artificial gravity: Toward a comprehensive countermeasure			
Wood, Scott	Naval Aerospace Medical Research Lab	Sensorimotor adaptation following exposure to ambiguous inertial motion cues			
Putchu, Lakshmi	JSC	Pharmacotherapeutics of intranasal scopolamine			

Team: Nutrition, Physical Fitness and Rehabilitation

PI Name	PI Organization	Proposal Title	Merit Score	ARS	Cat.
Wolfe, Robert	UTMB	Nutritional Countermeasures to Ameliorate Losses in Muscle Mass and Function			
Ferrando, Army	UTMB	Combined Effects of Nutritional and Exercise Countermeasures (Funded with Non-Core Dollars to NSBRI)			

Team: Smart Medical Systems

PI Name	PI Organization	Proposal Title	Merit Score	ARS	Cat.
Soller, Babs	University of Massachusetts Medical School	Noninvasive Measurement of Blood and Tissue Chemistry			
Thomas, James	The Cleveland Clinic Foundation	Echocardiographic Assessment of Cardiovascular Adaptation and Countermeasures in Microgravity			

Team: Technology Development

PI Name	PI Organization	Proposal Title	Merit Score	ARS	Cat.
Tai, Yu-Chong	California Institute of Technology	Handheld Body-Fluid Analysis System for Astronaut Health Monitoring			
Pisacane, Vincent	United States Naval Academy	MicroDosimeter Instrument (MIDN) System Suitable for Spaceflight			
Qin, Yi-Xian	SUNY- The State University of New York	A Scanning Confocal Acoustic Diagnostic System for Non-Invasively Assessing Bone Quality			
Maurer, Richard	Johns Hopkins University	Combined Ion and Neutron Spectrometer for Space Applications			
Charles, Harry	Johns Hopkins University Applied Physics Laboratory	Ground Based Measurement of Bone Loss in Astronauts Using AMPDXA GCS			

Pisacane, Vincent	United States Naval Academy	MicroDosimeter Instrument (MIDN) System Suitable for Spaceflight			
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Appendix J

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE



Annual Program Report: *Education and Public Outreach Team*

October 22, 2004

Team Leader

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EDUCATION AND PUBLIC OUTREACH TEAM PROJECTS

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Outreach Program for the Professional Development of Students and Teachers on Studies Related to Biomedicine in Outer Space

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National Space Biomedical Research Institute Teacher Academy Project

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From Outer Space to Inner Space: Sharing NSBRI Progress with the Community

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Secondary and College Education for the Next Generation of Space Life Scientists

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I. ABSTRACT

The Nation's education needs have been framed by the President's challenge to, "leave no child behind" and the 21st century workplace requirement for a science literate society. Such ambitions require bold vision and strong leadership for systemic change across the educational spectrum. NASA Administrator Sean O'Keefe has taken up this challenge, stating, "Education is part of our [NASA's] core mission." That mission includes the goal, "to inspire the next generation of explorers. . . as only NASA can."

The National Space Biomedical Research Institute (NSBRI) Education and Public Outreach Team has been working to achieve these directives through a coordinated, multi-institutional strategy that helps to meet our nation's education needs. This strategy is designed specifically to assist in educating the next generation of space biomedical researchers and to transfer the medical and biomedical findings of space research to the scientific community, the home and the classroom. The Team's mission is to communicate the significance and excitement of space life sciences to local, national and international audiences, while transferring and disseminating knowledge gained by the biomedical advances achieved by other NSBRI Research Teams. This mission currently is being accomplished through an integrated array of programs that focus on students and educators at all grade levels, as well as the general public. The Education and Public Outreach Team develops and implements activities that address the following four major goals:

- Design and conduct a variety of **teacher professional development** programs to help teachers understand space life sciences and change their practices and behaviors to improve the learning experiences they provide students.
- Develop **curricular materials** that span the educational continuum; are aligned with national science education standards; provide accurate, balanced, effective and inquiry-based instruction; and expand students' understanding of and interest in ongoing NSBRI research.
- Promote **educational access and career awareness** in bioastronautics research among high school and undergraduate students, as well as high school teachers.
- Increase **scientific literacy and public awareness** of the real-life impacts of NSBRI research through media, informal science activities, direct mailings and journal publications.

The Education and Public Outreach Team is comprised of seven primary partners: Baylor College of Medicine (BCM); Massachusetts Institute of Technology (MIT); Morehouse School of Medicine (MSM); Mount Sinai School of Medicine (MSSM); Rice University and The University of Texas Medical Branch (RU/UTMB); Texas A&M University (TAMU); and the University of Washington (UW). Twenty-seven other organizations and institutions—including state public school systems, public television and radio stations, state space grant programs and museums—are working with the Team to promote its mission and to ensure the widest possible dissemination of its products and programs.

The Education and Public Outreach Team is helping NSBRI to address the educational goals set forth by President Bush and Administrator O'Keefe. Hundreds of teachers and thousands of students have benefited from the Team's NSBRI-sponsored programs; and the public has been reached through television and radio news programs and national magazine articles. The Team's ongoing efforts are establishing NSBRI as a leading resource for bringing the excitement and importance of NSBRI space life science research into the nation's classrooms and homes.

II. INTRODUCTION

The mission of the NSBRI Education and Public Outreach Team is to communicate the significance and excitement of space life sciences to local, national and international audiences, while transferring and disseminating knowledge gained via the biomedical advances achieved by NSBRI Research Teams. This mission is being accomplished through an integrated array of programs focusing on students and educators at all grade levels, as well as the general public. Team goals are as follows:

- Design and conduct a variety of **teacher professional development** programs to help teachers understand space life sciences and change their practices and behaviors to improve the learning experiences they provide students.
- Develop **curricular materials** that span the educational continuum; are aligned with national science education standards; provide accurate, balanced, effective and inquiry-based instruction; and expand students' understanding of and interest in ongoing NSBRI research.
- Promote **educational access and career awareness** in bioastronautics research among high school and undergraduate students, as well as high school teachers.
- Increase **scientific literacy and public awareness** of the real-life impacts of NSBRI research through media, informal science activities, direct mailings and journal publications.

The Education and Public Outreach Team is comprised of seven primary partners: Baylor College of Medicine (BCM) in Houston, Texas; Mt. Sinai School of Medicine (MSSM) in New York, New York; Massachusetts Institute of Technology (MIT) in Cambridge, Massachusetts; Morehouse School of Medicine (MSM) in Atlanta, Georgia; Rice University and The University of Texas Medical Branch (RU/UTMB) in Houston and Galveston, Texas; Texas A&M University (TAMU) in College Station, Texas; and the University of Washington (UW) in Seattle, Washington.

A number of major organizations and institutions are working with NSBRI's Education and Public Outreach Team. Notable among these partners are (in alphabetical order): Aldine Independent School District, DeKalb County Public Schools, Atlanta Educational Telecommunications Collaborative, Inc., Atlanta University Center, Emory University Center for Behavioral Neuroscience, Fernbank Science Center, the nine Galveston County school districts, Georgia Institute of Technology-SECME Program, Georgia State Partnership for Excellence in Education, Harvard Medical School, Houston Independent School District, Johnson Space Center, New York Public Schools, New York Hall of Science, Space Center Houston, Spelman College, Texas Alliance for Science, Mathematics and Technology, Texas Rural Systemic Initiative, the Texas Statewide Systemic Initiative, and the Washington Space Grant program.

Synergy among individual project goals is achieved through the following four themes: teacher professional development; curriculum development; science literacy and public awareness; and access and career awareness. The following charts show how the teams work with each other.

NSBRI Education and Public Outreach Team Activities, 2003-2004

PI/PROJECT	GOAL ADDRESSED				PHASE 1	PHASE 2
	Teacher Professional Development	Curriculum Development	Science Literacy and Public Awareness	Career Awareness and Access	Planning Implementation Evaluation	Dissemination
Patrick J. Gannon <i>Defying Gravity: Enduring Life in Space</i>	Science Teacher Summer Workshops, Institutes	9th Grade Curriculum	Museum Exhibits Newsletter and Websites	Museum Exhibits Newsletter and Websites	Project Year 3	
Deborah L. Illman <i>Northwest Outreach Program on Space Biomedical Research</i>			NSBRI Magazine Stories; Science Communications Workshops	NSBRI Magazine Stories; Middle School "SciScape" Inserts	Project Year 3	
Robert James NSBRI <i>Teacher Academy Project</i>	Master NSBRI Teacher Program Teacher Workshops	NSBRI research related, inquiry-based classroom activities	Press releases and coverage of TAP teachers in their home states	TAP teachers interact with 4 NSBRI researchers on A&M campus and with NASA Astronauts and engineers	Project Year 3	NSBRI TAP activities presented at national and state conferences and at local level
Marlene MacLeish <i>Secondary and College Education for the Next Generation of Space Life Scientists</i>	Second year teacher activities and workshops	Problem-based Cases: 5-12 grade	NSBRI Film Archive	Undergraduate Summer Research Program	Project Year 6	Materials and activities presented at national and international levels.
Dava J. Newman <i>Space Biomedical Sciences & Engineering Curriculum and Outreach Project</i>		Undergraduate and Graduate Courses; K-12 Materials	Modular Knowledge Stations (Interactive Exhibit)	Modular Knowledge Stations (Interactive Exhibit)	Project Year 3	
Roland B. Smith <i>Outreach Program for Development of Students and Teachers on Studies Related to Biomedicine in Outer Space</i>	Year-long Program for 16 Secondary School Teachers	NSBRI Teacher-Developed Secondary Science Curriculum Units	Website	Teachers work with JSC astronauts and university research faculty	Project Year 3	Curriculum Materials Available
William A. Thomson <i>From Outer Space to Inner Space: Sharing NSBRI Progress with the Community</i>	Summer Workshops and Workshops at Professional Meetings	Three NSBRI-focused Teacher Units Completed (for Middle School)	Space Center Houston Activities; NASA Connect Broadcast; BioEd Online Website	Space Center Houston Activities; NASA Connect Broadcast	Project Year 6	Curriculum Materials Available

III. TEAM ACCOMPLISHMENTS, OCTOBER 2003-SEPTEMBER 2004

Baylor College of Medicine—From Outer Space to Inner Space: Sharing NSBRI Progress with the Community.

Baylor, an original partner of the NSBRI Education and Public Outreach Team, has continued to promote scientific literacy, access to biomedical careers, and public appreciation of space life

sciences research. during the 2003-2004 funding year. Below are selected highlights, activities and achievements for the 2003-2004 funding year.

- Created a prototype “virtual workshop” of Baylor’s NSBRI *Food and Fitness* module, which allows Baylor to reach teachers across the US. The virtual workshop, available on *BioEd Online* (www.bioedonline.org/workshops), was filmed during a live session with Houston teachers. It features a content overview by nutritionist Roberta Anding, MS (faculty member at Rice University and dietician to the Houston Texans football team), accompanied by video clips and downloadable PowerPoint slides and notes corresponding to each of seven student investigations in the module. After completing all components, teachers may take a post-test and receive credit for contact hours of professional development recognized by the Texas Education Agency.
- Collaborated with JSC Bioastronautics Education and Outreach and the LaRC Office of Science Education, allowing Baylor to reach much larger audiences with NSBRI-related teaching resources. During Fall 2003, Baylor’s NSBRI *Food and Fitness* module served as a template for a NASA Connect™ broadcast on healthy eating and exercise. Work is underway for Baylor and NASA to collaborate on adapting additional modules in Baylor’s *From Outer Space to Inner Space* series into at least five more television broadcasts with supporting web-based resources. The existing program, “Better Health From Space to Earth” will be aired again on PBS on April 2005. It may be viewed online at:
http://connect.larc.nasa.gov/programs/2003-2004/better_health/index.html.
- Conducted 12 NSBRI unit workshops and presentations for approximately 725 educators, representing more than 17,400 students. Additional teachers received professional development in BCM’s NSBRI curricular materials at Space Center Houston. BCM’s activities included invited presentations at the following meetings.
 - National Association of Biology Teachers, Portland, OR, October 9, 2003
 - NASA Biological and Physical Research Enterprise, Education and Outreach Annual Conference, New Orleans, LA, January 7, 2004
 - NSBRI Annual Investigator Retreat, Conroe, TX, January 16, 2004
 - National Science Education Leadership Association, Atlanta, GA, March 31, 2004
 - National Science Teachers Association, Atlanta, GA, April 3, 2004
- Filled requests from around the US, including Space Center Houston at Johnson Space Center, for more than 5,768 copies of BCM’s NSBRI Teacher Guides and more than 2,195 NSBRI classroom posters.
- Incorporated BCM’s NSBRI curricula into other programs:
 - used in two, one-week summer (June) professional development Institutes for a total of 42 elementary and middle school teachers in Houston.
 - used in a two-week summer (July) professional development program, funded by the National Science Foundation, for 100 lead science teachers in the Houston Independent School District (BCM’s NSBRI curricular units were presented and disseminated to all participating teachers);
 - implemented in workshops for (and disseminated to) eight elementary school teachers participating in BCM’s Science Education Leadership Fellows program, funded by the Howard Hughes Medical institute;

- incorporated into professional development for (and disseminated to) 15 high school life sciences teachers as part of Baylor's GK-12 program, funded by NSF;
 - featured in a six-week after-school science club for Houston students, funded by the National Heart, Lung and Blood Institute;
 - used with six premedical students participating in Baylor's six week summer enrichment program, known as the Honors Premedical Academy, funded by The Robert Wood Johnson Foundation; and
 - used with 13 high school students participating in the summer component of Baylor's Bioscience Inspiration and Opportunities for Students program, funded by NIH.
- Continued developing the fourth unit in the *From Outer Space to Inner Space* series.
 - Wrote an article entitled "An Approach to Improving Science Knowledge About Energy Balance and Nutrition Among Elementary and Middle School Students." This article, which discusses BCM's most recent NSBRI Teacher Guide, *Food and Fitness*, has been published by the journal, *Cell Biology Education*. Summer 2004 (number 3), pages 122-130.
 - Wrote a second article focusing on Baylor's NSBRI work, which has been accepted for publication in *Acta Astronautica*. This article is entitled Increasing Student Learning Through Space Life Sciences Education.

Massachusetts Institute of Technology – Space Biomedical Sciences and Engineering Curriculum and Engineering Curriculum and Outreach Project

- In Fall 2003, Professor Newman taught a graduate course at MIT for 15 students: "Space Biomedical Engineering and Life Support" (<http://paperairplane.mit.edu/16.423J>). Guest lectures were provided by NSBRI investigators, Oman, Merfeld, Mark, and Heldt.
- Space Biomedical Engineering and Life Support educational outreach materials developed under the name, "Spacerciser," have been used to teach hundreds of middle and high school students. Teacher manuals have also been developed (<http://dorffman.mit.edu/spacerciser/>).
- In Spring 2004, Professors Merfeld and Oman led graduate a course at MIT for 12 students: "Sensori-Neural Systems: Spatial Orientation from Vestibular End Organs to Behavior and Adaptation." Other NSBRI investigators involved include Young and Wall.
- Completed physical design and construction of "Knowledge Station," created to promote public awareness of NSBRI and NASA educational outreach efforts. Compiled education materials from varied sources, including MIT, NSBRI and NASA. Completed storyboards, layout and interface between users and information. Professional multimedia implementation is in process. The Knowledge Station is scheduled to be deployed at the MIT Learning Laboratory in Fall 2004, the Museum of Science in Boston, and the Peabody Essex Museum.
- Conducted/was accepted for the following two presentations:
 - Newman, D.J., Marquez, J., Brown Wagner, E., Trotti, G., Merfeld, D., Oman, C. "Integrating Engineering Education and Research Through Space Exploration," ICEER 2004 International Conference on Engineering Education and Research Progress Through Partnership, Olomouc, Czech Republic, June, 2004.

- Newman, Marquez, Wagner, Merfeld, and Trotti. "Explore Space: Integrating Space Biomedical Engineering Education and Research." To be presented at and published in the proceedings of 55th International Astronautical Congress. Paper No. IAC-04-P.3.09, (October 2004).

Morehouse School of Medicine—*Educating the Next Generation of Space Life Scientists.*

Teacher Institute – Teacher Outreach Activities

- MSM-NSBRI teachers and staff participated in planning a three day retreat (October 14-16, 2003), entitled *NASA Corporate Recruitment Initiative*, attended by Administrator O’Keefe and NASA Education Chief, Dr. Adina Loston. A full NSBRI Education and Public Outreach exhibit was launched for the event (approximately 500 attended).
- MSM-NSBRI hosted Dr. Charles Lloyd, JSC-Bioastronautics Education Chief, for a two-day visit (December 3-5, 2003) to MSM, Fernbank Science Center, and the Sci Trek Museum (10 teachers attended).
- MSM-NSBRI hosted a conference on April 1, 2004, to plan a 2005 NASA–NSBRI Big City Immersion for Atlanta. The conference was attended by 30 science educators representing various NASA entities, Atlanta University Center science programs, Atlanta-based science museums, radio, television and elementary/secondary science educators.
- MSM-NSBRI hosted a Presidential Appreciation Award ceremony for Dr. Jeffrey Sutton, NSBRI Director. The award was presented by MSM President, James Gavin, III on April 1, 2004. Approximately 20 people attended.
- MSM-NSBRI hosted approximately 150 participants from across the US at a Distinguished Educators Award Ceremony on April 1, 2004. Dr. Jeffrey Sutton, NSBRI Director, and Ms. Bonnie McClain, Education Chief, NASA Headquarters, presented awards to six educators for contributions to science literacy and space life sciences education. Recipients included Dr. Walter Sullivan, MSM Vice President; Dr. James King, Institute Professor of Space Sciences, Morehouse College; Dr. Carolyn Randolph, President of the National Science Teachers Association.
- MSM-NSBRI Teacher Institute presented five sessions—The Journey to Mars, Striking a Happy Balance, Sleep and Circadian Rhythms, The Cardiovascular System in Space, and Pig Heart Dissection Laboratory—at the 52nd Annual National Science Teachers Association Convention, held from April 1-9, 2004, in Atlanta, GA. Fifteen teachers attended.
- MSM-NSBRI Program Director addressed students from across the nation at the 8th Annual National Minority Health Professions Foundation Symposium at Morehouse School of Medicine in Atlanta, GA, on April 7, 2004. Approximately 100 students attended.
- MSM-NSBRI Program Director represented NSBRI on a 20-person NASA delegation to Scotland, at the invitation of the Scottish government, from June 13-21, 2004. The delegation visited six cities and spoke to approximately 1,500 students across Scotland.

- NSBRI Teacher Institute teachers used materials to design inserts for the problem-based case, *Bobby's Beat*.
- MSM-NSBRI director awarded the 2004 Murphy - Woman of Distinction Award from the University of Western Ontario-Brescia College, in London, Ontario, Canada, for contributions to space life sciences education. Approximately 200 attended.

Summer Research Program

- Four undergraduate students completed a ten-week intensive research program including: circadian biology laboratory research; weekly journal club presentations; school-wide poster session/final oral presentation (approximately 50 attended); science writing workshop; core laboratory skills; and ethics training.
- Updated longitudinal database on 32 past students for program outcome measurements.
- Conducted exit interviews and evaluation for summer students.
- Attended the 2004 JSC Internship Program Banquet on July 21, 2004, in Houston, Texas.

Archives

- Initiated update of MSM web site to include activities for the previous two years.

Mt. Sinai School of Medicine—*Defying Gravity: Enduring Life in Space*

- Presented a workshop entitled “Astronaut Well-being on Trips to Mars” at the regional meeting of National Science Teachers Association South Midwestern Area Convention, Kansas City, MO, November 13 - 15, 2003.
- Presented a workshop entitled “Compelling Space Biomedicine Themes Used to Enhance Biology Learning” at the national meeting of National Science Teachers Association in Atlanta, GA, April 3, 2004.
- Guest of NASA [BPR Educational Outreach] booth at the national meeting of the National Consortium of Teachers of Mathematics in Philadelphia, PA. This was a collaboration with BPPE, Chuck Lloyd and Monica Trevathan. Mt. Sinai’s NSBRI tableside poster, entitled “Math Missions to Mars And Beyond,” with its compelling subtitles, “You Can Be a Mathstronaut Tonight” and “Get a Math Feel for The Milky Way Galaxy,” enticed many teachers to ask for more details. A hands-on exercise in which we recruited teachers to experience the fascinating phenomenon in which the body shrinks in height throughout the day, and then expands up to 2 inches overnight, was met with considerable disbelief. This simple but powerful exercise caused the 57 teachers who responded the next day (77% of those recruited) to recognize how this experience would excite and motivate their students. They believed students would gain inspirational knowledge of both NASA’s pursuits and high-level NCTM standards-based math through this activity.
- To beta test for awareness and understanding of curriculum material by students in grades 3-5, Mt. Sinai presented modified components of three of its NSBRI modules, “Thinking Big,” “Smells to Mars,” and “BonE Voyage” to 80 students at PS 198 in New York City.

- At the invitation of Mr. Dan Porter, Patrick Gannon spoke to a group of 45 middle and high school teachers attending an NSBRI professional development workshop at the Morehead Planetarium (University of North Carolina, Chapel Hill) on February 7, 2004. The two-hour keynote lecture and hands-on lab session focused on the importance of the perceived chemical senses, smell and taste, for life on Earth and in space. It was based on the Mt. Sinai NSBRI module, "Smells to Mars."
- NSBRI's Defying Gravity: Embracing Life program now has an active Performance Patch available to participants in the Girl Scouts Council of Greater New York. This patch is earned by conducting activities corresponding to all Scout levels: Daisy, Brownie, Junior, Cadette and Senior. The new patch is based within the "Women with Wings, Girl Scouts Conquer the Sky" initiative.
- Mt. Sinai's Defying Gravity program (www.defyinggravity.net) was featured in a series of articles published in the "Parents Know" venue, the home of *Big Apple Parent*, *Queens Parent*, *Westchester Parent*, and *Brooklyn Parent*; a group of NYC-based monthly publications with a readership of 140,000 parents, teachers and general public (<http://www.parentsknow.com/>). In the March, 2004 issue, Defying Gravity appears in a two-page spread (pp 92-3). The feature article, entitled "Space on Earth: Educating the Next Generation of Astronauts," describes the Bon-E Voyage lesson, based on osteoporosis research.
- The premier issue of Voyage (July 1, 2004), published by the British Interplanetary Society (<http://www.bis-spaceflight.com/voyage.htm>), featured an article about astronaut David Williams' visit [December, 2003] to the Defying Gravity program at Mount Sinai School of Medicine to speak to more than 600 students, teachers and the public.

Rice University/University of Texas Medical Branch—Space Science Education

Teacher Institute for the Advancement of Space Science Education

- Conducted two NSBRI related workshops at the Regional National Science Teacher Association Conferences on November 15, 2003, in Kansas City, MO and on December 6, 2003 in Reno, NV. The workshops, entitled "Bringing the Final Frontier To Your Classroom," highlighted the outstanding secondary science activities developed by teachers participating in the NSBRI Teacher Institute.

2003-2004 Teacher Academic Year Follow-up Activities

- September 20, 2003: UTMB Regional Science Teachers Conference. Summer curriculum products were disseminated.
- November 22, 2003: Curriculum-focused activities on Protein Crystallization in the High School Lab, Modeling a Closed Environment, Snell's Law and Refraction, and Recycling Water in Closed Environments. Space resource materials were distributed to participating teachers.
- March 10, 2004: Grant Writing Workshop (optional) for teachers.
- March 24 – 25, 2004: Transferring Curriculum Components to the Web.

- May 10, 2004: End-of-program reception for Year Three teachers. Teachers from Years One and Two also were invited to participate in this “Sharing Of Best Practices” event.
- We continue our efforts to publish a workbook of curriculum products developed by teachers during the three-year NSBRI grant. Each instructional activity will contain information for teachers, student activities, assessment, and adaptations and extensions for implementation.
- Completion of review by science curriculum specialists to validate the accuracy of science concepts, mathematics connections, instructional strategy and alignment with National Science Education Content Standards.

School District Partners

Houston Independent School District, Aldine Independent School District, Galveston County school districts, including: Clear Creek Independent School District, Dickinson Independent School District, Galveston Independent School District, High Island Independent School District, Hitchcock Independent School District, LaMarque Independent School District, Moody Independent School District, Santa Fe Independent School District, and Texas City Independent School District.

School Partners – Receiving NSBRI Curricular Resources (27)

Austin High School – Houston, TX; Ball High School – Galveston, TX; Bellaire High School – Houston, TX; Clear Creek High School – League City, TX; Clear Lake High School – Clear Lake, TX; Clear View Alternative High School – League City, TX; Davis High School – Houston, TX; Drew Academy – Houston, TX; Furr High School – Houston, TX; High School for the Engineering Professions – Houston, TX; High School for the Performing & Visual Arts – Houston, TX; Jackson Middle School – Houston, TX; Jones High School – Houston, TX; Lamar High School – Houston, TX; LaMarque High School – LaMarque, TX; Lee High School – Houston, TX; MacArthur High School – Houston, TX; Madison High School – Houston, TX; Milby High School – Houston, TX; Reagan High School – Houston, TX; Sam Houston High School – Houston, TX; Santa Fe High School – Santa Fe, TX; Sharpstown High School – Houston, TX; Space Center Intermediate School – Clear Lake, TX; Sterling High School – Houston, TX; Stevenson High School – Houston, TX; Welch Middle School – Houston, TX

Informal Science Partner: Space Center Houston

Government Supported Partner: Johnson Space Center

Texas A&M University—Teacher Academy Program (TAP)

- 79 Tier I TAP teachers reached 1580 Tier II teachers.
- Tier I TAP teachers reached 2,443 students (996 male, 944 female, 257 African Americans, 46 Asian Americans, 191 Hispanic, and 9 Native American (not all teachers provided demographic information)).
- TAP teachers conducted 40 presentations around the US.
- TAP faculty and staff gave NSBRI-related presentations at the following events.
 - Texas State Science Teachers Conference (CAST) Houston, TX, November 2003

- International Space Station Educators' Conference, Houston, TX, February 2004
- National Science Teacher Association National Conference, Atlanta, GA, April 2004
- Katie Bynum, thesis defense plus presentation at NSTA in Atlanta, GA, April 2004.
- Earned the following honors and awards.
 - Presidential Award for Excellence in Science Teaching-Hawaii, Pascale Pinner, Hilo, HI
 - Milken Educators Award for Excellence in Science Teaching, Melissa Miller, Farmington, AR
 - Finalist, Educator Astronaut Program, Jennifer Sinsel, Wichita, KS
 - Finalist, Educator Astronaut Program, Teri Rowland, Sheridan, WY
 - Ciba Specialty Chemicals Exemplary Middle Level and High School Principal and Teaching Award, Jennifer Sinsel, Wichita, KS
 - TEAM of the Year Award from Arkansas Middle School Association, Jennifer Milligan, Little Rock, AR
 - Mississippi Middle School Science Teacher of the Year, Kay Williams, Magnolia, MS

Earth Based Applications of Research Project

- A series of NSBRI TAP lessons has been created and refined during the three years of the project. They all have been field tested by participants and shared with Tier II teachers. Now, they have been uploaded onto a web page with other components of the project so that they will serve as an easily accessible resource for teachers seeking hands-on activities to teach NSBRI research related concepts.

The University of Washington—Northwest Outreach Program on Space Biomedical Research

- Developed articles on space and space biomedical research for publication in *Northwest Science & Technology (NWS&T)* magazine, disseminated to readers in the Northwest and beyond. Articles included the following.

	Author
Winter 2004: "Young Minds, Fresh Ideas: NSBRI and Washington Space Grant help train tomorrow's scientists"	Jeff Wolfe, Master's student, Department of Technical Communication, U Washington
"NASA Appoints Oregon Professor to Direct Space Biology Research"	Melissa Phillips, Department of Biological Structure, U. Wash.
"Bathing in the Big Bang: Astronomers Unravel Mysteries of Our Universe"	Marcel Agueros, Ph.D. student, Department of Astronomy, U Wash.
"Soccer Ball, Doughnut, or Bagel? MSU physicist at the center of debate over shape of the Universe"	Annette Trinity-Stevens, Director of the News Service at Montana State University-Bozeman
Autumn 2004: "An Interview with Astronaut Janet Kavandi: UW alumna and former Boeing engineer talks about her experience at NASA"	Jeff Wolfe, Master's student, Department of Technical Communication, Univ. of Wash.
Winter 2005, in preparation: Astronautical Frontiers: Modeling Human Physiology for Space Exploration. This news story covers the International Astronautical Congress 2004 (IAC04), Vancouver, B.C, Oct. 4-8, 2004. Highlighted is a symposium led by Martin Kushmerick and Ronald White: "Mathematical Modeling and Physiological Simulation in Preparation for Human Exploration."	Ben Raker, <i>NWS&T</i> editorial assistant and alumnus of the UW science writing program

- A poster presentation on "Media Coverage of Engineering," presented at the 2004 Gordon Research Conference on Science and Technology Policy, presented our results on content analysis of science and technology, including space coverage, in the *New York Times* Science Times section.
- Recruited two students who completed the 2004 summer research program, working with Professor Marty Kushmerick, bringing the total to date of ten summer students. This year, students Lauren Palmer and Sara Van Nortwick worked with Kushmerick on techniques to quantify muscle metabolism as part of a larger effort to model human biological processes mathematically and develop new medical tools for use in space and on Earth. The students' work was supported by the NSBRI in combination with funding from the Washington NASA Space Grant program, the Mary Gates Foundation, and an Intel Diversity Grant.
- Initiated development of a science communication survey for NSBRI teams; results to be received during fall 2004.
- Printed NSBRI's display ad in each issue of the magazine published during the project.

IV. TEAM PLANS FOR NEXT 12 MONTHS

This item is not applicable to the Education and Public Outreach Team, as Team funding under the current grant has ended.

Marlene Y. MacLeish, EdD
Team Leader
NSBRI Education and Public Outreach Team

Date

Appendix K

NSBRI 2004 Summer Internship Program

INTERNS	SUPERVISOR	ASSIGNMENT	DATE
Andrew Abercromby University of Houston aabercromby@uh.edu	Dr. Bill Paloski 281-244-5315	Neurosciences Laboratory Bldg. 37/Room152A	5/17/04 - 8/20/04
Danielle Blauw University of Rochester Blauw@bme.rochester.edu	Dr. Mill Reschke 281-483-7210	Neurosciences Laboratory Bldg. 272	6/1/04 - 8/25/04
F.J. Haran University of Houston fjthree@hotmail.com fharan@uh.edu	Dr. Jacob Bloomberg 281-483-0436	Neurosciences Laboratory Bldg. 272/Room 107	5/17/04 - 8/20/04
Jason Hoggan United States Air Force Academy c05jason.hoggan@usafa.edu	Dr. JoAnna Wood 281-244-5524	Behavioral Laboratory Bldg. 272	5/22/04 - 6/25/04
Jismi Jose Rensselaer Polytechnic Institute jose@rpi.edu	Dr. Linda Shackelford Scott Smith 281-483-7100	Bone and Mineral Laboratory Bldg. 261/Room 136	5/24/04 - 8/17/04
Julie Litzenberger Stanford University jlitze@stanford.edu	Dr. Neal Pellis Dr. Alamelu Sundaresan 281-483-4343	Cellular Movement and Signal Transduction Laboratory Bldg. 37/Room 1082	6/27/04 - 9/3/04
Damien Mockus University of Texas-Tyler fitnesslon1@hotmail.com	Dr. Don Hagan 281-244-1122	Exercise Physiology Laboratory Bldg. 37/160	5/17/04 - 8/20/04
Felicity A. Pino University of Missouri-Columbia fapc94@mizzou.edu	Dr. Deb Harm 281-483-7222	Neurosciences Laboratory Bldg. 272/Room 106	5/24/04 - 9/8/04
Mae Sattam University of Texas-Austin wmsattam@mail.utexas.edu	Dr. Todd Schlegel 281-483-9643	Neuroautonomic Laboratory Bldg. 272/Room 114	6/7/04 - 8/13/04
Justin Seret Rensselaer Polytechnic Institute jseret@alum.rpi.edu	Dr. Kathy Johnson- Throop 281-483-0387	Medical Informatics and Healthcare Systems Group Bldg. 37/126	6/1/04 - 8/25/04
Scott Sheehan George Washington University School of Medicine sheehans@gwu.edu	Dr. Todd Schlegel 281-483-9643	Neuroautonomic Laboratory Bldg. 272/Room 114	6/7/04 - 7/30/04
Amanda Tamm UT Dental Branch-Houston Amanda.L.Tamm@uth.tmc.edu	Dr. Linda Shackelford Scott Smith 281-483-7100	Bone and Mineral Laboratory Bldg. 261/Room 136	6/1/04 - 7/23/04
Leah Zidon Truman State University lczidon@yahoo.com	Dr. Jacob Bloomberg 281-483-0436	Neurosciences Laboratory Bldg. 272/Room 107	6/1/04 - 9/1/04

Andrew Abercromby
104 Garrison
University of Houston
4800 Calhoun Road
Houston, TX 77204
aabercromby@uh.edu
Tel: 281-770-0046

August 28th 2004

Dear Dr. Sutton,

Thank you again for another invaluable 3 months experience of working in the Neurosciences Laboratory at JSC! My NSBRI internship in 2003 in the same laboratory was extremely helpful in allowing me to integrate quickly into the lab and begin my research study with little delay. This quick start was also facilitated by a series of discussions I had with my supervisor, Dr. Paloski, prior to beginning the internship. Dr. Paloski agreed that I would be the principal investigator on a research study I had proposed, which would investigate the biomechanical and neuromuscular responses to vertical whole-body vibration during standing:

Exposure to short periods of Whole-Body Vibration (WBV) at particular frequencies during standing has been shown to elicit acute and chronic force and power increases in the muscles of the lower body. It has been suggested that these physiological adaptations are a result of increased muscle activity elicited through a muscle spindle-modulated reflex. Studies on seated subjects have shown that the transmission of vibration through the body and subsequent muscle activity varies following changes in seated posture. The body posture that is associated with maximum muscle activity during standing whole-body vibration has not been established. The harmful effects of exposure to whole-body vibration at various frequencies have also been documented, prompting the creation of standardized methods of measuring and evaluating whole-body vibration exposure by the International Organization for Standardization (ISO 2631-1). Harmful effects of short-term exposure to whole-body vibration can include motion sickness and discomfort, while more serious health effects such as low back pain are thought to be associated with long-term exposures.

The proposed study will measure changes in muscle activity and the transmission of vibration through the body over a range of body postures. The results will provide insight into improving the effectiveness of whole-body vibration as a neuromuscular training method and will also quantify the vibration stimulus with respect to the ISO standards.

The study involved collaboration between the Neurosciences Laboratory, the Countermeasure Evaluation and Validation Project (CEVP), the University of Houston, and the University of Texas Medical Branch (UTMB). Whole-Body Vibration has been investigated as a potential countermeasure to, among other things, disuse-related muscle atrophy and osteoporosis. As such, its potential use of WBV as a countermeasure to these conditions during spaceflight is worthy of investigation.

The study required the integration of four channels of electromyography (EMG), two accelerometers, eight infra-red kinematic position markers and the use of two different WBV platforms. The first two weeks of my work dealt with the administrative and physical challenges of moving the requisite equipment between institutions and between laboratories. I then custom-made several cables such that all signals could be sampled simultaneously in a single data acquisition unit. Concurrently with the hardware and software integration tasks, I applied for and received expedited approval to perform the study from the JSC Committee for Protection of Human Subjects, subject to the satisfactory completion of a Test Readiness Review.

For the protocol design, I worked with Bill Amonette of CEVP and we also consulted on a number of occasions with statisticians Dr. Al Feiveson and Dr. Hsi-Guang Sung whose input was valuable in ensuring that the data we would collect would be balanced and would lend itself well to statistical analysis.

Following the completion of extensive documentation of the study rationale, protocol, and hazard analysis, a Test Readiness Review was arranged and approved the study with only minor adjustments to the protocol.

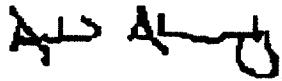
A total of sixteen test subjects were scheduled through the JSC Human Test Subject Facility. Bill Amonette was involved in the data collection process, which lasted four weeks. The interaction with human test subjects and the experience with data collection was one of the most valuable experiences of the summer. I learned a lot from working with Bill, who has less technical experience than I do, but more experience with human test subjects and data collection. I feel we complemented each other well and enjoyed working with him.

Following the completion of data collection, the remainder of the summer was spent writing data analysis programs which would make sense of the several Gigabytes of data we had collected! This work will continue throughout the coming semester during which I will be continuing my graduate studies at University of Houston. My security clearance is to be extended such that I have year-round access to JSC allowing me to continue working on the analysis of this data and possibly begin work on other projects. There has been some interest in the use of vibration at JSC since I performed this study and I am optimistic that further collaborative studies investigating the effects of WBV might be possible.

On a personal level, I feel the summer was extremely successful. I learned a lot about the research process and have the makings of one or two publications to show for it. There are a few things that I could have done better in retrospect but I consider that an inevitable part of the learning process. I was happy to have the independence and trust given to me by Dr. Paloski while also being able to work with many other people. It is the ability to meet and work with the people at JSC - including other interns - that makes these internships so valuable to me.

In the coming year, I hope to begin my dissertation research (which may be vibration-related) and I am aiming to complete my PhD by December 2005. I would then like to participate in the NSBRI post-doctoral program and perform further research at JSC possibly investigating physiological responses to vibration.

My sincere thanks to you and to all of the staff at NSBRI for allowing me the opportunity to spend another summer at JSC.

A handwritten signature in black ink, appearing to read 'A. Abercromby'.

Andrew Abercromby

Danielle Blauw
NSBRI Internship Report
9/30/04

I first want to start out saying that having the opportunity to be an intern for NSBRI is one of the greatest experiences I've ever had. It was a great learning experience for me with all of the lab work I did. I was able to pull from several areas of my education and use it over the summer. In Dr. Reschke's lab, I had the opportunity to work on two projects. I have a strong background in biomechanics, which I got to apply in the functional stretch reflex project that I worked on. I used my background in neuroscience and the vestibular system for the gaze holding project I did. I will describe both projects and my participation in them. The work I did on both is very much related to the work in the lab, as these two projects are the current main focus of the Reschke lab.

I will first describe the first project I started working on, called "Modification of Eccentric Gaze Holding." This current project is studying the ability to maintain fixation on eccentric targets while either upright or supine. Both the horizontal and vertical planes were considered. The subject would be securely fastened in a chair, which can be locked in an upright or supine position. An LED board would be placed at a fixed distance in front of the subject, parallel to the coronal plane. There were 5 LEDs on the board, which were positioned at center, left, right, up, and down. The left and right targets were placed so that the subject would need to make a 20-degree eye movement from center to view them. The up and down targets were placed at 15 degrees. The subject would wear goggles with infrared cameras to record eye movements. All experiments were completed in total darkness so that the subject could see only an LED when illuminated. The study I participated in is a shadow study for data collected at the Naval Aerospace Medical Research Lab (NAMRL) in Pensacola, FL. In Pensacola, the same experimental set up was mounted to a 25 ft radius centrifuge. The exact protocol was reproduced at JSC, without centrifugation, to be sure that any results seen in Pensacola were because of centrifugation and not the time commitment or any other characteristic of the trials that the subjects were asked to do. During each trial, the LEDs would flash in a randomized order for 20 milliseconds. The subject was asked to look at the target and maintain fixation on

where they think the target was once it turns off for about 20 seconds. We are studying the ability to maintain fixation, and the characteristics of movement as the eye drifts away from the target position. Specifically, we are trying to calculate the null position of the eye, and the time constant at which the eye moves to this position. The null position is the position that the eye would be at in a completely relaxed state. This is not necessarily anatomical zero, and we would like to quantify this. The null position also may change as a function of the direction and size of the gravity vector through the head, which is why we are observing behavior in upright and supine positions. Over the course of the summer, my contributions to achieving this have been collecting shadow data at JSC.. I assisted in data collection for 12 subjects. I then digitized all of data we collected and entered it into Matlab. Once I got the data into Matlab, I would run scripts to calculate the null position and time constants for all of the data collected at JSC. I also did a large amount of the data processing for data collected at NAMRL.

I will now describe the second project I worked on, called "Functional Stretch Reflex". This is one of several studies being conducted as part of the bed rest project. Subjects that enroll in the bed rest study lay in bed at a 6-degree head down angle for 60 days continuously. It is a ground-based analogue to long duration microgravity exposure. My lab is studying the effects of bed rest on muscle reflexes, particularly in the gastrocnemius. We are using electromyography (EMG) to study the changes in the muscle reflex over the duration of the study. We will be recording from the medial and lateral heads of the gastrocnemius muscle, the medial and lateral soleus muscles, and the anterior tibialis. During our data collection, the subject will be lying supine with his foot securely fastened to a pedal, which is connected to a rotating stepper motor. This allows us to provide resistance to movement and the ability to measure the forces that the subject generates while responding to the tapping stimulus. The stimulus is provided by a force-transducing hammer striking the pedal, causing the achilles tendon to stretch, thus evoking a reflex. We will be measuring the change in reaction time and magnitude of the response. We predict that we will see an increase in the time to respond, along with a decrease in magnitude. The first campaign started in the last week of my internship. My involvement this summer on this project was to help prepare for the start of the study. I helped with organizing and developing

procedures for testing. Many times I volunteered to be the practice subject for developing protocols.

As for the future, this experience became an immediate part of the plan. Two weeks ago, I became a full time employee in the Reschke lab. The main reason that I wanted to have an internship this past summer is that I wanted an experience outside of academia. I had decided that being a professor in academia was no longer a path that I wanted to take, which left me not knowing what my other options could be. I had been unhappy in the lab I was working in back at home, and was questioning whether I want a PhD at all. My advisor was very supportive of my confusion, allowing me to take an internship to figure out what I want to do. I still haven't completely figured it out, but I did find that if I do pursue a degree that I would like to transfer to a different school and join a different lab. In the meantime, since I had such a wonderful summer experience here, and being that there is plenty of work for me to do, I have decided to become part of the neuroscience lab at JSC while I look into transferring to a different school.

My Summer Experience in the Neurolab

I spent my summer working with Dr. Jacob Bloomberg in the Neuroscience Motion Laboratory. Dr. Bloomberg is currently the PI of a NSBRI project called "Understanding full-body gaze control during locomotion." I worked closely with Dr. Bloomberg and other members of the laboratory staff on this project. The project addresses risks caused by spaceflight such as post-flight imbalance, vertigo, and visual instability. I learned how to monitor, address, and predict the occurrence of such risks, and some possible prevention measures.

The ability to stabilize gaze control or simply vision while walking requires that three systems function and coordinate together: the eye-head, head-trunk, and the lower limbs. One of the goals of our laboratory was to study how these systems interact by altering or perturbing one system and observing how this change affects a subject's gaze stabilization during walking. It is hoped that this kind of research will lead to a better understanding of how the eye-head, head-trunk, and lower limbs coordinate to stabilize gaze during walking. Once the proper coordination pattern between body segments is established, it will aid in the development of improved post-flight testing procedures to evaluate an astronaut's gait and to determine the effectiveness of proposed countermeasures.

During the course of the summer I was exposed to various subprojects that were occurring simultaneously throughout the lab. I became familiar with projects that focused on the effects of unloading on gait;; the effects of changes in visual acuity on

gait;; the effects of virtual reality immersion on gait; and changes in gait pre- and post-spaceflight. In addition to being exposed to all of these projects, I became familiar with all of the hardware and software that is used in the collection and analysis of the data. It was my personal project to analyze the amount of variation in the gait cycle. To do so I had to research the topic of variability in human gait extensively, learn how to use MatLab to write software for the necessary analysis, and improve my communication skills so that I could effectively seek help for problems I encountered.

The first problem I faced was determining what exactly “variation in gait” is and the practical methods for ascertaining the amount of variation. In the gait cycle, movement consistency is crucial as it determines our ability to make intentional modulations of the stride to control balance. If movement during the gait cycle is not fairly consistent it can cause us to fall when we are exposed to even the slightest perturbation to our gait pattern. Recent research has focused on the variability of the time between consecutive heel strikes of the same foot, which is commonly referred to as a stride interval. Humans are able to modulate their stride intervals via various parameters that directly control the amount of temporal and spatial variability in gait patterns. Normal variation in the gait cycle is about 5 % and anything more will adversely affect our ability to maintain balance.

Stride intervals have shown fluctuations from one interval and display “noisy” variations that are not uncorrelated brown noise.. These noisy variations exhibit long-range power law correlations that are indicative of fractal processes. It is not unheard of for human systems to exhibit fractal properties, and it is well-known that the human heart displays fractal properties, but it was surprising to see this finding in the gait cycle.

Researchers have observed how this fractal property differs among various populations. There is a higher fractal index in a normal population, indicating that the stride intervals are uncorrelated and show more variability. Young children, the elderly, and populations with movement disorders all have shown a smaller fractal index, indicating less variability. What this means is that populations with smaller fractal indexes have less variability and are thus less stable.

It is known that astronauts post-flight have shown difficulty walking and are less stable. It was the goal of my research to determine if there is a difference pre- and post-flight in the amount of stride interval correlation variability. In order to do this, I had to code in Matlab an analysis technique called detrended fractal analysis. Unfortunately, the analysis did not work as we had a limited number of stride intervals and fell short of the number we needed for the analysis. However, going through the research process on a space-related topic using a technique I had never heard of was an amazing experience. I plan on using this technique to assess the variability of head pitch correlations collected from astronauts pre- and post-flight for my dissertation. This technique will be perfect for that data set as there are more than enough data points for the analysis. I have also been offered access to pre-existing data, and to the lab itself, for my dissertation process.

If I had not been chosen to take part in NSBRI's summer internship program and spent two-and-a-half months being exposed to the research in this lab and the minds that work there, I would not be on the path I will be starting this academic year. I now have a dissertation topic, access to a prestigious lab that is outside of my university, and access to brilliant scientists. I have also had exposure to research on the effects of space flight that I may have never otherwise had, which has affected how I view this type of

research. Overall, I had a great learning experience at NSBRI—one that I consider one of the best experiences of my life.



**DEPARTMENT OF THE AIR FORCE
34th TRAINING GROUP
USAF ACADEMY, COLORADO 80840**



20 July 2004

MEMORANDUM FOR KATHY MAJOR, NSBRI

FROM: C1C Jason Hoggan

SUBJECT: Trip Report for CSRP at the Johnson Space Center through NSBRI.

1. **PURPOSE:** While acting as an intern under Dr. JoAnna Wood in the Behavioral Laboratory at the Johnson Space Center in Houston, Tx, my primary purpose was to assist in data analysis, graphical preparation for presentation and papers, and literature reviews. I acted in this capacity from 24 May until 25 June 2004, during which time I focused most of my attention on Dr. Wood's most extensive data base, entitled "The Gold Standard." This data set was created by hundreds of personnel who participated in 100-day (or other time period) Antarctic expeditions over the past 15 years. The set attempted to quantify the psychological and psychophysiological effects of relative isolation in extreme environments over time, including internal as well as interpersonal factors.

2. **TRAVELER:** C1C Jason Hoggan, USAFA/CS-08

3. **ITINERARY:** NASA Behavioral Laboratory at Johnson Space Center, 24 May 04 – 25 Jun 04

4. **DISCUSSION:** During the first few days of my internship with Dr. Wood, I was required to gain a better understanding of multi-level processing, which she uses quite frequently to analyze her data because it is categorized into week, person, and month levels in the set. After reading some information on this type of processing, I also read two of the studies that Dr. Wood had

already completed regarding the data set, which gave me a better understanding of the type of information she was analyzing and the types of trends she was trying to discover. After this introductory period, I began my functional role by spending a few days to create a 3-dimensional graphical representation of data for a presentation regarding personality traits such as enthusiasm (F) and energetic drive (Q4) as defined by the 16PF on perceived cognitive ability against weeks. I also developed a panel-variable representation of changes in leadership perceptions over time, according to the different individual's responses at each station. Using a program called Textsmart, I then analyzed the subjects' use of humor in their open-ended responses, attempting to find a link between those aforementioned psychological factors and the use of either negative or positive humor in the stations. Finally, I made an observation regarding the differences between what individuals reported as negative experiences versus what they considered stressful experiences, and this observation generated another line of exploration regarding the open-ended responses. This analysis took almost a week of work, and generated much useful information which will probably result in a small paper from Dr. Wood in the near future.

5. CONCLUSION/RECOMMENDATIONS: This summer research opportunity was a great experience for me, as well as gave me a better understanding of the inner workings of NASA and other similar organizations. It also gave me a greater appreciation of the Behavioral Sciences and Statistical disciplines, as well as their practical applications in the world of psychological research and development. Not only did I become an active member in a very close team at the Johnson Space Center, but I was also given a number of other great opportunities while I was there. For example, I was given the opportunity to tour the facilities, including the Command Center, and I was also able to listen to Bob Curbeam, a Naval officer and astronaut, speak about and show video clips from his recent flight up to the space station, during which he installed the new ISS Laboratory. Finally, I learned quite a bit about how to use the many different software programs for statistical analyses, including SPSS (in much more detail than we use at the Academy), HLM, MLWin, Adobe Illustrator, Excel, and Textsmart. Although Dr. Wood will probably not be working at JSC much longer, I recommend that the Academy keep NSBRI and JSC as contacts for the Behavioral Science department for future summer research opportunities, for I have gained invaluable experience and knowledge from my time there. In terms of my future plans, I hope to explore a different type of data analysis as a special agent in the Air Force

Office of Special Investigation after I graduate in May of 2005. I hope to use my experience with NSBRI to help me achieve this goal, as well as help me perform that function with greater accuracy and precision.

6. Thank you for your time and consideration.

Respectfully,

JASON C. HOGGAN, C/LtCol, USAFA
Squadron Commander, Cadet Squadron 08

Jismi Jose
Rensselaer Polytechnic Institute

NSBRI Summer Intern 2004
May 24-August 17, 2004

Working for the National Space Biomedical Research Institute at NASA's Johnson Space Center has been one of the most amazing experiences of my life. I have learned so much about NSBRI and the space program during my stay here.

I have always been curious about space and astronauts but my interest really grew after I started working at the Johnson Space Center. At JSC, I was involved in the research challenges that scientists are faced with, and I was able to contribute to the space program. I had never known how much goes into exploring space and sending astronauts into space. Having the opportunity to work at JSC has opened up numerous doors for me to see things I never thought I would be able to see, to meet some amazing people who have had an impact on my life, and to learn more than any textbook could teach me.

The title of my project was "Exercise Manual for Increasing Bone Mineral Density in the Wrist, Spine, Hip, and Heel". I collaborated with another NSBRI intern to create a report of exercise loads and efficacy for preventing bone loss and increasing bone density by comparing the exercise peak net load vectors for the wrist, spine, hip, and heel to the loads of exercises for bone density reported in past literature.

Research from over the years has shown that astronauts lose a significant amount of bone mineral density (BMD) during long duration space flight. The goal of my research was to analyze previous studies and record their findings in a table. The table of information is a concise summary of published BMD and exercise studies. The point of the table is to illustrate the wide range of results and protocols of exercises. By analyzing the studies, we are trying to focus on the exercises that link particular activities to

changes in BMD in the specific regions of the body. The exercises should build bone density in younger individuals and decrease the rate of loss in older individuals (it may increase BMD in older individuals in the absence of endocrine changes).

As part of my project, my partner and I took pictures of the exercises being performed by both of us. The exercises believed to strengthen the bone in the wrist, spine, hip, and heel are the following: bilateral leg press on machine, deep squat with weights above shoulder, deep squat with machine, donkey calf exercise, finger pushups against the floor and against the wall, good morning exercise, modified squat with weights above shoulder, modified squat with Smith machine, Romanian deadlift, shallow angled single leg press, single leg press on machine, single leg Romanian deadlift, wrist curls, and reverse wrist curls.

These pictures along with the exercise manual will be able to help individuals especially at risk of osteoporosis to take the appropriate measures to prevent bone loss and increase BMD. It will also contribute to the research going on in the Bone and Mineral Lab and Exercise Physiology Lab investigating countermeasures to strengthen muscles and prevent bone loss in astronauts during space flights.

My internship at NASA was further enhanced with tours that we were taken on visiting places such as the Neutral Buoyancy Lab, Ellington Field, Moon Rock lab, and International Space Station mock-ups among others. The lectures held by various astronauts, the ISS Manager and CAPCOM were all great times to learn so much about the space program and have really inspired me to pursue a career in the space and biomedical field.

I will be a junior at RPI this coming year and will continue pursuing my bachelor's degree in biomedical engineering. During my project I was introduced to bone physiology, muscle physiology, and biomechanics. This will greatly help me in my upcoming school year when I have to take a human physiology class and a biomechanics class. Working on this project helped me to apply what I've already learned in school to real world challenges. I would love to participate in another internship involving space and the biomedical sciences; so if given the opportunity, working for NSBRI next summer is a possibility that I am extremely excited about.

From this great experience at JSC I gained more knowledge about NASA's endeavors, along with understanding and taking back with me to college the research pertaining to my major. I am so grateful to have had this opportunity to contribute to the important scientific research that seeks solutions to health concerns facing astronauts on long missions and that also benefits individuals on Earth suffering from parallel health conditions. Interning for NSBRI has introduced me to the challenges NASA faces and I hope that I can stay involved in their research efforts in the manned space field throughout the years.

**National Space Biomedical Research Institute
Summer Internship Program**

Julie Litzenberger

**Final Report
Summer 2004**

September 3, 2004

My Research

During my ten-week tenure in the National Space Biomedical Research Institute (NSBRI) summer internship program at NASA Johnson Space Center (JSC), I helped to conduct experiments dedicated to analyzing the effects of microgravity on human biological systems. The summer internship greatly enhanced my education by providing me with exposure to the type of research that is conducted through the NSBRI at NASA JSC, by giving me the opportunity to learn a variety of molecular biology techniques, and by allowing me to experience and gain knowledge in immunology research, thereby diversifying my biological background. I had the privilege of learning from an excellent mentor, Dr. Alamelu (Lalita) Sundaresan, and collaborating with students from a variety of backgrounds. Experience gained through this internship has strengthened my understanding of standard molecular biology laboratory procedures, and will prove invaluable throughout the remainder of my graduate and professional careers.

Working in the Cellular Movement and Signal Transduction Lab (CMSTL) at NASA JSC, I participated in two major research initiatives. My primary project focused on examining the effects of simulated microgravity on gene expression of human immune cells. Additionally, a portion of my time was devoted to a bone project in the CMSTL, in which bone cells were exposed to modeled microgravity. Microgravity was imitated in the laboratory using the NASA Bioreactor, a rotating wall incubator that spins cells in a closed chamber at a constant velocity, so that the cells remain in perpetual free-fall. This allows cells to develop in a three-dimensional construct, a behavior that closely mimics what has been observed in the microgravity environment of space and "*in vivo*". The NASA bioreactor provides scientists with a tool to study the effects of modeled microgravity on human physiological systems at a cellular level, and I was fortunate to gain experience using the device throughout my summer internship in the CMSTL. I will discuss my projects in further detail below.

In June 2004, peripheral blood mononuclear cells (PBMC) were isolated from human blood samples in the CMSTL. Two sets of cells were rotated in the NASA Bioreactor for one hour duration. Two sets of ground controls were maintained at 1 g, and one time control was isolated at the zero-hour time point. We used cells from this experiment throughout the remainder of the

summer, running molecular biology experiments to understand the genetic effects of simulated microgravity on the human immune system. Preliminary BCA protein assays were used to determine the protein concentration in each of the five samples. Using protein assay data, sample volumes were calculated and used to run gel electrophoresis and Western Blot analyses. I worked closely on this project with Jason Conover, an undergraduate bioinformatics student from Baylor College in Waco, Texas, who taught me a number of molecular biology techniques. In my ten weeks in the CMSTL, Jason and I performed fourteen Western Blot analyses on the PBMC samples, which were used to examine differences in protein expression in microgravity samples versus ground controls. Prior to my experience in the CMSTL, I had never run a gel electrophoresis or a Western Blot. By the end of the summer, I felt practiced and capable using these techniques in molecular biology applications. I compiled a set of laboratory protocols for growing cells in the rotating wall vessel used in the NASA Bioreactor, as well as for performing a BCA Protein Assay and a Gel Electrophoresis Analysis with a Western Blot detection system. My research in the CMSTL provided me with thorough exposure to these laboratory procedures, and I am confident that this experience will greatly enhance my future educational endeavors.

In addition to my work in the CMSTL at NASA JSC, I also had the opportunity to visit collaborators at the Bioinformatics Center of the University of Texas Medical Branch (UTMB) in Galveston, TX. A supercomputer at the UTMB is used to perform a gene array analysis of experimental samples from the CMSTL. From this data, researchers can determine which genes are up-regulated or down-regulated as a result of exposure to simulated microgravity.

The second project that I was involved in through the CMSTL is still greatly under development, and aims to “establish *in vitro* 3D culture models of osteoclasts, osteoblasts, and their co-culture to study bone resorption under ‘normal’ and ‘microgravity analog’ culture conditions.”

Ultimately, these tissue models will be used in bone mineralization and resorption experiments to study microgravity-induced bone loss. While participating in the project, I and two other students from the University of Houston helped to maintain osteoblast and osteoclast cell cultures and learned various cell labeling and counting techniques. This work strengthened my knowledge in bone remodeling strategies, as well as allowed me to practice my skills using sterile cell culture procedures that I will certainly draw upon during my future research endeavors.

My Education

In May 2002, I received a Bachelor of Science degree in Civil and Environmental Engineering from Tufts University in Boston, Massachusetts. If I could have looked two years down the road, to find myself in a molecular biology laboratory at Johnson Space Center, I would have been in complete disbelief. I have found a field that I am passionate about and a research area that I plan to devote the rest of my life to. In September, I am returning to Stanford University to commence my second year of graduate school. I will receive my master's degree in Mechanical Engineering, with a concentration in Biomechanics, in December 2004. I hope to begin working towards my Ph.D. in Bioengineering at Stanford University in the spring of 2005.

My educational goals have transformed significantly over the past few years, and my internship at JSC through the NSBRI has played a major role in solidifying my decision to pursue a doctorate degree in Bioengineering. I plan to focus my graduate research on understanding the effects of long duration spaceflight on human physiological systems, specifically the role that microgravity plays in bone mass loss. Prior to beginning graduate school, I worked full time in the Bone and Signaling Laboratory at NASA Ames Research Center for one year. I conducted research primarily with laboratory animals, and gained a comprehensive exposure to working in a wet lab. In my first year of graduate school, I performed experiments to understand the effects of fluid flow on calcium signaling pathways in bone cells. I learned a variety of sterile cell culture techniques and observed several molecular biology experimental procedures. It was not until this summer, however, that I had the opportunity to run my own gel electrophoresis analyses and Western Blot detections. I became familiar with these common laboratory procedures and greatly strengthened my overall knowledge of how scientists use these techniques to test hypotheses regarding biological systems. Additionally, I diversified my research experience this summer by straying from bone cells and working instead with immune cells. I had the opportunity to conduct research with Dr. Alamelu (Lalita) Sundaresan, who is a lead immunology researcher at NASA JSC. I learned not only practical molecular biology laboratory procedures from Dr. Sundaresan, but also the more theoretical aspects of conducting scientific experiments and the pragmatics of running an innovative and productive government laboratory.

My summer internship with the NSBRI was an exceptionally valuable experience, and I would recommend the program to other graduate and undergraduate students without hesitation. The NSBRI lies at the cross-roads between engineering and biology in human spaceflight. To fulfill the ultimate goal of human exploration, the physiological adaptations of long duration spaceflight must be understood and mitigated. It was a privilege to conduct research with the NSBRI. My experience has helped to solidify my future research goals in gravitational biology and has exposed me to the various types of research that are available through the NSBRI and at NASA JSC.

Acknowledgements: I would like to give special thanks to the following people who helped to make my summer experience so incredible.

Dr. Alamelu (Lalita) Sundaresan, NASA JSC
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Kim Sandhoff, NASA JSC and UH
Dr. Antonios Kyparos, NASA JSC
Dr. Neal Pellis, NASA JSC
Kathy Major, NSBRI
Lauren Hammit, NSBRI
Iris Ali, NSBRI
Dr. Jeffrey Sutton, NSBRI
The students of the 2004 NSBRI summer program

Thank you.

Summer Internship Report

Felicity Pino

During the three months I worked at the NASA Johnson Space Center, I worked in the Neurosciences Department, in the Preflight Adaptation Training (PAT) lab under Dr. Deborah Harm. I was fortunate enough to be assigned the completion of my own research study: an experiment analyzing the effects of virtual reality exposure on human motion sickness and posture.

My first task after discussion with researchers in the PAT lab was to propose a project definition for experiment approval. Although the outline to be turned in followed a simple paradigm, presenting project goals and details forced me to define and organize the plans for myself. Once the project was approved through NASA-JSC, I was ready to request subjects from the Human Test Subject Facility (TSF). The TSF selected a requested number of subjects (15), gave the PAT lab a list of those names, and I was responsible for contacting those subjects for participation.

I presented the research project and goals, in layman's terms, to gain potential subjects' interest to participate in my study. Those available and willing were then scheduled for testing. The actual testing portion of my project included performing the actual experiment on the participating subjects, collecting data, and organizing and analyzing collected data.

To observe the effects of virtual reality exposure, each subject was placed in a virtual reality environment, comprised of an interactive "video game" in which the subject was given a task of placing and moving highlighted objects, both quickly and accurately. Exposure duration was thirty minutes.

The first effect of exposure I was observing as a tester was on human posture. Each subject underwent postural tests, consisting of a platform on which he/she would stand upright. As the platform perturbed his/her posture--either sliding forward, sliding backward, or tilting toes-upward—a score depicting his/her maintained balance (variation in center of gravity) would be given (EquiTest®, Neurocom®). I would compare posture scores from before, immediately following, and one hour following exposure to the virtual reality environment. The second factor of interest was motion sickness as a result

of virtual reality exposure. Motion sickness symptoms were self-reported by each subject, by means of questionnaires.

Upon completion of data collection and analysis, I presented for the Neurosciences Department my experimental results and conclusions. The project I had worked on was simply a pilot study, from which the need for further pursuit of these topics was to be determined. A similar study was being simultaneously pursued in the PAT lab. Whereas my study was observing motor control skills (maintaining posture during platform perturbations), the other study observed sensory skills (maintaining posture without normal visual and somatosensory feedback) affected by virtual reality exposure.

Both types of skills are of interest to NASA scientists, as humans exposed to spaceflight are under sure reconstruction of vestibular functioning. If human subjects exposed to virtual reality have similar effects in vestibular, sensory, and motor function as astronauts exposed to spaceflight, then virtual reality exposure can be used as an analog to space exposure. If unpleasant vestibular effects of space exposure (e.g., dizziness, motion sickness) can be lessened by adapting to the space environment, the space environment analog (virtual reality) might be useful in preflight adaptation training. For this reason, the PAT lab is undergoing studies, similar to mine, to determine the effectiveness of virtual reality training as an “altered environment” analog.

From performing this study, I gained more knowledge in the general field of research. I practiced patience, a necessary quality in research, I developed hypotheses and conclusions, and I got the chance to work with human subjects, a new-to-me aspect of research I highly enjoy! I feel I contributed to the PAT lab researchers by competently accomplishing a long-anticipated goal.

By working at NASA-JSC, I got hands-on experience in a lab setting and with proper procedures for setting up research pursuits. I further developed my independence as a researcher. I have a better idea of my personal strengths, weaknesses, interests and hesitations; I know more now what it is I desire to put into and take out from my research, scholastic, and professional duties.

I can apply the skills I learned and practiced this summer to my future pursuits. I soon begin an MS/PhD program in biomechanical engineering at Stanford University,

where I am eager to explore various research topics and avenues. I am excited to also pursue a teaching experience while in graduate school. The topic to which I am most attracted during my graduate training, whether that be in an industrial or academic setting, is what I will ultimately pursue as a career.

Mae Sattam

The National Space and Biomedical Research Institute has not only offered me a “position as a project intern for Baylor College of Medicine,” as it says in my acceptance letter, but it has given me an opportunity to work on cutting edge research at Johnson Space Center with incredibly intelligent and inspirational people. I have also made friends, who have all given me a great perspective on what this country has to offer, from across the nation, and I was also given the opportunity for many chances to inspire others like myself through various press releases. My second summer interning under the direction of Dr. Todd Schlegel through NSBRI has far surpassed my high expectations and, consequently, has flown by in a blink of an eye.

As I began my summer, my previous experience with Dr. Schlegel’s research allowed me to jump straight into the projects working with high frequency QRS electrocardiography projects, instead of spending many weeks studying the basic background of electrocardiographs. My main goal has been to write a protocol to diagnose and manage a parasitic disease endemic in Latin America, called Chagas’ disease. Cardiac involvement is the most prominent manifestation in Chagas’ disease, and *Trypanosoma cruzi*, the causative agent of the disease, infects well over 16 million people in Latin America. Sudden arrhythmic heart failure, manifested from Chagas’ disease, is the leading cause of cardiovascular mortality in widespread areas of Latin America. I studied much literature about this disease and about the electrocardiograph parameters used to help diagnose and manage it. My application of the knowledge I have gained over the past years about this software played a significant role in order to compose the protocol that will be used by doctors and researchers in Latin America.

While I was working on the protocol, my co-workers in the laboratory aspired to improve the heart rate variability program with additional installments to the heart rate turbulence portion. By assessing the variability of the heart rate, one may evaluate the patient's ability to regulate its heartbeat through neuroautonomic function. If this function is not controlled as expected by a healthy individual, this measurement may indicate cardiac dysfunction and that the patient is at-risk for sudden cardiac death. With the high-frequency QRS software, an online analysis of heart rate variability calculates and reports parameters and trends related to both stochastic and deterministic heart rate variability as well as to non-invasive baroreflex sensitivity for a measurement of heart rate turbulence. The improvements made onto this program helped to further define in the protocol the features of the software that may be used in order to aid Chagas' patients. In addition, by writing this protocol, this research team at NASA is merely one step closer to introducing the high frequency QRS software as a diagnostic tool to all hospitals where it will aid millions with cardiovascular conditions and diseases.

From the eyes of an observer looking in on the lives of the NSBRI interns at Johnson Space Center, the interesting research with their mentors and fascinating lectures from various NASA personalities, such as Astronaut Don Petit and CAPCOM Ginger Kerrick, are definitely apparent. However, the absolute feature of my internship has been working and learning from the amazing interns NSBRI has chosen this summer. The incredible diversity within this group of interns range from medical students to graduate students, and for the undergraduates, these new friends have given great advice on their future careers. As for the present and future, my passion will always remain with the improvement of life on earth and with the exploration for possible life outside this planet.

Therefore, this year, I plan to apply for a summer internship program with the AMES Academy at the NASA site in California, where I will be focusing on astrobiology. The other internships that I plan on applying for are at a mental institute in Thailand, at Kennedy Space Center through the Space Life and Sciences Training Program, or at a research internship in the Texas Medical Center. As one may observe, I enjoy exploring many types of programs around the country and even across the globe, but without my first internship through NSBRI, I would have never realized about the infinite number of places and programs to which I may contribute my knowledge and zeal for space and medicine.

Justin Seret
NSBRI Internship Report

During my NSBRI summer internship in 2004, I worked under the supervision of Dr. Kathy Johnson, with the Medical Informatics and Healthcare Systems (MIHCS) group. This was quite a change of pace for me – as a biomedical engineer, I had not been exposed to the field of medical informatics before. Medical informatics deals with the study, invention, and implementation of structures and algorithms to improve communication, understanding, and management of medical information. The primary projects that the group was working on were the WorkIT and Logger databases. These databases were used to log medical events (e.g. flight sickness, changes in breath/heart rate, etc.) and other data from medical devices (often concerning the functionality of the devices). I was to perform a rudimentary evaluation of the databases. I sent comments on ease of use, effectiveness of features/layout, and functionality to the database designers. After the first couple of weeks, I realized that my skills would be better suited for a more engineering-oriented team. Fortunately, Dr. Johnson was able to provide me with some other options on teams to work with.

During my third week I began working with the Smart Healthcare Management Systems (SHMS) Team. I was able to transition over smoothly through M.G. Sriram, who worked with both MIHCS and SHMS. SHMS was focusing mostly on evaluating and developing wireless sensing technology. I started with SHMS by helping team member Darryl Parker with tests on several wireless location tracking systems (including Ekahau, Versus, and Radianse). These tests were done at NASA's Advanced Integration Matrix (AIM), to assure the accuracy and reliability of the devices. Wireless tracking

systems may one day be used in the International Space Station or on Mars missions in order to tell Mission Control where astronauts are at any given time. This information would be useful in many medical scenarios. For example, if an astronaut had trouble breathing in a certain location, Mission Control (or anyone monitoring the astronaut) could mark the location as being potentially hazardous (perhaps having a high concentration of carbon monoxide or other toxic gases).

Next, I took a look at the variety of other sensors that the group worked with. These included multi-functional sensors, such as Crossbow's MICA2 "mote", and single-function sensors (such as Invocon's wireless CO2 monitor). MICA2 motes are essentially low-cost, low-power computers that are about the size of a deck of cards. They can be equipped with a variety of sensors (temperature, light, electrochemical, vibration, stress, etc.), and can form ad-hoc, self-healing networks. I learned quite a bit about this technology over the summer, and would be very interested in working with it in the future. However, I worked more closely this summer with Invocon's wireless CO2 monitor. As the name suggests, the monitor determines the carbon dioxide levels in any given location, and transmits them wirelessly to a computer. The levels are then displayed on Invocon's software, where they can be interpreted. I performed a variety of tests on the monitors, including distance-to-signal-failure, stability and accuracy (tested against Industrial Scientific's Class III portable CO2 monitor), and AIM testing (to test consistency in the performance of two side-by-side units). The overall objective of my testing was to see whether Invocon's quality claims were met, and make a preliminary determination on whether or not the units would be suitable for use on the International Space Station (ISS). I concluded that the units were less accurate and less stable than

Invocon claimed, and that they would require further development before they went on for formal approval for use on the ISS.

My work over the summer helped to increase my interest in designing and developing biomedical instrumentation for space flight applications. During my previous summer with NSBRI, I focused on computer simulations of the musculoskeletal system. This summer, I was able to work with the hardware itself, and I realized that I would really like to continue to work with devices which include embedded control elements (such as Crossbow's MICA2 motes). These combine hardware and software systems, hence allowing me to combine my interests and experience. One day, I hope to improve the health and lives of astronauts (and people everywhere) through development of smart medical technology.

7/30/2004

When I try to summarize what this experience has meant to me, I can't help but think back to how oblivious I was to what the internship was when I applied. To be honest, I had no idea what I was getting myself into when I took this internship. I knew it would involve medical research, and it was going to be at NASA-JSC, and that was good enough for me. Being a lifelong fan of the space program, I wasn't going to miss an opportunity to work at NASA. It's safe to say however, that I never imagined that the experience would prove to be as rewarding as it has. This has been an outstanding internship, both educationally and personally.

I'm currently a medical student at George Washington University in Washington D.C., and at GW, you're strongly encouraged to do something meaningful with your only free summer of your medical career. While I wasn't sure exactly what they meant by "meaningful", I was pretty sure that my original summer plan of doing part-time roofing didn't qualify. I looked into research, having no significant experience to speak of, and I stumbled across the NSBRI website by sheer luck, via a google search for NASA Space Biomedical Research Internship. I was lucky enough to have found the program four days before the application deadline, and was even luckier to have been selected.

Upon selection, I was placed in a lab at JSC working under Dr. Todd Schlegel. I was tasked to contribute to one of his projects involving real-time analysis of heart-rate variability in determining degree of cardiac dysautonomia and its link to mortality. The project is based essentially on studies which have shown that a decrease in heart rate variability (HRV) is linked to an increase in sudden cardiac failure. The exact mechanism is unknown, but the correlation exists. Therefore, the ability to detect minor trends toward invariability could lead to early detection of impending cardiac failure, with the possibility of intervention. The project itself is a software program which integrates with another related program Dr. Schlegel developed, measuring high-frequency QRS complexes in real-time with a similar purpose.

The project works by recording patient data from an ECG, and analyzing in real-time the high-frequency component of the waveform for abnormalities, as well as the variability of the RR-intervals themselves. My role in this project was to find a way to automatically filter out the effects of premature ventricular contractions (PVC's), to make the system more robust. Should a PVC occur during an otherwise normal ECG recording, the computed variability of the average RR-interval would be drastically effected. Since we're looking for a lack of variability as a positive indicator for disease, the introduction of a PVC would represent an abnormal irregularity, resulting in a net average which is more irregular (implying more "normal") than it actually is. Thus, to prevent false negatives, PVC's must be eliminated from the computations.

I was tasked to eliminate the PVC's from calculations, and I honestly couldn't have asked for a better assignment, for several reasons. First, to meet this goal, I would have to become proficient with electrocardiography, and familiar with the manifestations of pathology represented on the ECG. These skills have served to complete the picture of cardiac physiology only glossed over previously, and will undoubtedly give me a huge advantage going into my second year of medical school. Secondly, this project has given me much needed experience in doing in-depth background research. Before I began this project, I didn't fully comprehend how research was conducted, or reported. I now feel

much more comfortable with medical literature. Lastly, and foremost, given my background in software engineering, this assignment put me in a position where I could really get involved and make a significant contribution. I somewhat expected that I would be doing a very scattershot domain of work during this internship. I expected to do either a lot of smaller, more menial assignments, or a perhaps contribute in a very small way to a project too large to really understand or appreciate. I feel my work on this project has been both large in scope and impact on the project. PVC rejection should prove to be extremely valuable, and as such, I feel I've made a very positive contribution to the program. In terms of work satisfaction, I couldn't be happier.

Working on this project has also helped me come to the realization that I would most enjoy having research make up a significant part of my medical career. I'm currently applying to graduate programs, focusing on aerospace physiology, in hopes of pursuing a PhD. I understand that there are several investigators affiliated with NSBRI in some of the research areas I would be interested in. I would welcome the opportunity to continue my affiliation with the NSBRI in that capacity. This experience has played a huge role in solidifying my desire to pursue research as part of my medical education.

The only aspect of this internship that I'm not happy with was how lucky I had to be to have found it. That may sound counter-intuitive, but I had no idea this program existed until I practically typed the name of the program into a web search-engine. Being a huge lifelong fan of the space program, I really felt that if anyone should've known about this before-hand, it should've been me. The fact that I didn't, means that the word isn't really getting out as well as it could. I'm not sure if it's a question of insufficient advertising, but I can't imagine it would be hard to find applicants to this program if people knew about it. I'm not sure exactly how to fix this problem either. Since I've been here, I've seen countless instances where NSBRI staff have worked with newspapers and radio stations to try to get the word out about the program. Hopefully, this program will get the publicity it deserved, because it's really too valuable to be kept a secret.

Finally, I want to sincerely thank everyone at NSBRI for allowing me this opportunity. This internship has been nothing but positive. I really can't express how valuable this experience has been, and I would do it again in a heartbeat.

Sincerely,

Scott Sheehan

Amanda Tamm

NSBRI Summer Internship Project Overview

Over the summer I worked in the Bone and Mineral Lab at Johnson Space Center in contribution of the project titled Exercise Manual for Increasing Bone Mineral Density in the Wrist, Spine, Hip, and Heel. I worked on this effort in collaboration with another NSBRI intern, Jismi Jose, under the supervision of Dr. Linda Shakelford, who supported our efforts through long distance communication as she was called to active duty for the military. Together, Jismi and I worked to produce a report of exercise loads and efficacy for preventing bone mineral loss and increasing bone density by comparing the exercise peak net load vectors for the wrist, hip, spine, and heel to the loads of exercises for bone density reported in current literature. This exercise manual will be beneficial before long-term spaceflights for those crew members who have low bone mineral density (BMD) as well as members of the general public with low BMD.

We began our project with a thorough literature review of all studies conducted involving specific exercises and their effects on both bone mineral density and muscle hypertrophy. Weight-bearing exercises as well as non-weight bearing exercises were both reviewed. Literature was especially noted if positive effects on BMD were seen among adolescents and if BMD was maintained among adults and elderly. Upon analysis of a wide variety of publications, we organized summaries of the material, which provided information needed to begin construction of the exercise manual. We were introduced to the use of free body diagrams for further analyzing the forces and moments placed on specific joints while a muscle is loaded during a particular exercise. From the literature and free body diagrams, it was

concluded that certain vectors and loads indeed strain bones in such a way that would improve bone mineral density with greater efficacy than others. With these analyses, we assembled an exercise protocol which best-emphasized bone growth while taking into account injury management. Photographs depicting our recommended protocol for each exercise were included in the manual for further description.

The summer internship experience at Johnson Space Center was truly a pleasure and has left a lasting impression on me. Although Dr. Shakelford was not there to address many of our concerns immediately, as communication was limited to mostly email, progression of the project persisted and the amount that I learned was significant. Throughout my internship, I gained a better understanding of the role of life science issues in the space program. I was able to add to my growing expertise in bone physiology and have especially gained further understanding of the interaction of multiple forces acting to load bone and thus, promote remodeling. I also enjoyed this unique opportunity to study exercise physiology and work in close association with the Exercise Physiology Lab at JSC.

I have several semesters of schoolwork remaining that will go towards completing my DDS degree. There are number of paths I may ensue following completion of school including involvement in academics, research, private practice, or most likely a combination of the fore mentioned. An active interest in various capacities is definitely the spice of life in my opinion and I hope to pursue any future challenging endeavors. I know that my interest in long- term space flight issues will especially continue to grow and I anticipate future project involvement with the space program.

Leah Zidon, Summer 2004

The past summers of 2003-4 have been both unique and enjoyable learning experiences. I have worked for the National Space Biomedical Research Institute as a project intern in both the Human Environmental Factors Office (2003) and the Human Adaptations and Countermeasures Office (2004) at the Johnson Space Center in Houston, TX. The summer of 2004 has been the more enjoyable of the two because I was working with neuroscience researchers, a field that better fit my interests than my previous internship. As an exercise science graduate, I had some exposure to neuroscience before arrival, but have learned much more since I have been involved with the neuroscience laboratories at JSC.

Within the neuroscience group, I worked with numerous types of projects. One of the main concepts I learned this summer was the neurovestibular system. The neurovestibular system is a series of sensory connections that gives indication about body orientation in regards to the environment. I studied many of the diverse aspects of this system. Because I was not involved with one particular laboratory, I worked with researchers on various studies that dealt with many distinct areas of neuroscience as a whole. I enjoyed the variety and learned much more information than I would have by working in one exclusive area. Each study dealt with a different part of the human neurovestibular system. I discovered the complexity this system offers to the human body and how much humans depend on it daily.

I feel that I gained much more this summer than I would have in any classroom. Some areas of neuroscience I had encountered in various studies were virtual reality, motion sickness, vestibulo-ocular reflex input, eye movement, human movement, dynamic visual acuity, posture stability, environmental vestibular adaptation and sensorimotor adaptation during locomotion.

Currently, starting in early September, I began working for Wyle Laboratories as a researcher on a study that I helped to conduct during the end of my internship this summer. The researchers are looking at the effects of immersion into a virtual reality environment on coordination and posture. This summer, I would test the subject's posture before the VR task, immediately after, and 1,2,4, and 6 hours later. Future work will involve testing hand-eye coordination and gaze fixation before and after the virtual reality task instead of the posture test.

In this lab, I began work with a head-mounted virtual reality device. Subjects played a game in which they pick up objects (with a 3-D mouse) at one end of the computer-based "room" and bring them through a series of random pathways to the other side of the "room." The virtual reality program simulates the subject looking around the room. If they move their head, the image on the screen moves the opposite direction. This task lasts for a half an hour or an hour, depending on random selection.

I have also used a dome with a 180-degree projector in which the subject will perform the same virtual reality task as he/she would have with the head-mounted device. The projector also has a program that is an image of the space

station. It requires input from a "space ball" in which the user has 6 degrees of freedom to "float" around the station. The user's goal is to find various switches and click on them with a 3-D mouse. The goal is to use this for training the crew in the future for adaptation purposes. The current virtual reality study may support this idea.

The experience that I have gained from my internship with NSBRI has given to me a new outlook on my future. Within the next couple years I plan to go back to school in a health-care related field. I am not sure exactly which field I would like to study, but I am leaning toward a career in physical therapy, with an emphasis in neurovestibular rehabilitation. I have been intrigued by neurovestibular science and rehabilitation and plan to incorporate my experiences at the Johnson Space Center with NSBRI and with Wyle Laboratories into my potential career.

Appendix L

Note: Document Altered to Remove Merit Scores

**NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE
EDUCATION AND PUBLIC OUTREACH PROGRAM**

PROPOSAL SELECTION SUMMARY FOR NSBRI-RFP-04-02

October 1, 2004

On May 24, 2004, the National Space Biomedical Research Institute (NSBRI) released an open solicitation, NSBRI-RFP-04-02, requesting proposals for "Expansion of Education and Public Outreach Activities." The call contained two opportunities. There was a request for proposals (RFP) for the Institute's K-16 Education and Public Outreach Program and a RFP opportunity for a Phase I (definition phase) Graduation Education Program. The deadline for applications was July 28, 2004.

Twenty-six applications were received to the K-16 portion of the RFP, with one application subsequently withdrawn by the principal investigator. Six applications were received to the Phase I Graduation Education opportunity. Proposals to both opportunities were peer-reviewed by an external panel (see attached) and scored for merit as described in the RFP. The proposals, merit scores (0-lowest, 100-highest) and peer-review comments were then discussed in executive session with the Institute's External Advisory Council (EAC), NSBRI Director and Associate Director, on September 22, 2004. The EAC recommended that the four highest-scoring K-16 proposals be selected, with the additional recommendation that these projects have a monitored evaluation component and meet national standards. The EAC recommended that the two highest-scoring Phase I Graduation Education proposals be funded.

The recommendations of the EAC were followed and the selected projects are listed below.

K-16 Education and Public Outreach Program

Marlene MacLeish, Ed.D.

Education for a Diverse World of Space Explorers and Scientists

Morehouse School of Medicine 3 years

Gary Coulter, Ph.D.

Improving and Expanding E/PO in Space Biomedicine: Emphasis on Informal Education

Colorado Consortium for Earth and Space Education 3 years

William Thomson, Ph.D.

From Outer Space to Inner Space

Baylor College of Medicine 3 years

Roland Smith, Jr., Ed.D.

Space Biomedical and Life Sciences Curriculum by and for Teachers

Rice University 3 years

In the letters of award for the K-16 Education and Public Outreach Program, special language is included notifying recipients that a workshop on evaluation and standards will be hosted by NSBRI during the first year of the awards. Program evaluation and adherence to national science standards for education are important elements of the grants (as stated in the RFP, p.5).

Phase I Graduate Education Program

Laurence Young, Sc.D.

Graduate Education Program in Bioastronautics – Phase I

Massachusetts Institute of Technology

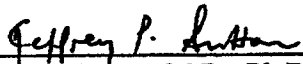
1 year

Joanne Lupton, Ph.D.

A Graduate Education Program Focusing on Space Life Sciences

Texas A&M University

1 year



Jeffrey P. Sutton, M.D., Ph.D.
Director, NSBRI

10/1/04
Date

Attachment: Peer Review Panel Roster

RFP NSBRI-04-02
Review Panel Members

Reviewer's Name	Reviewer's Organization
Wayne Sukow, Ph.D.	National Science Foundation
Jason Briggs	Optical Society of America
Bruce A. Jackson, Ph.D.	Boston University School of Medicine
Barbara Laval	University of California, Los Angeles
Carolyn Narasimhan, Ph.D.	DePaul University
Gail Nordmoe, Ed.D.	Sacred Heart University
B.L. Ramakrishna, Ph.D.	Arizona State University
Marcus W. Shute, P.E., Ph.D.	Tennessee State University
Daniel L. Wulff, Ph.D.	University at Albany
M. Jean Young, Ph.D.	MJ Young & Associates

Appendix M

Note: Document Altered to Remove Merit Scores

**NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE
EDUCATION AND PUBLIC OUTREACH PROGRAM**

**POSTDOCTORAL FELLOWSHIP SELECTION
SUMMARY FOR NSBRI-RFP-04-01**

October 1, 2004

On April 14, 2004, the National Space Biomedical Research Institute (NSBRI) released an open solicitation, NSBRI-RFP-04-01, requesting proposals for "Postdoctoral Fellowship Applications." Applications were due June 30, 2004.

NSBRI received 29 applications, with one submission subsequently withdrawn by the applicant. Applications were reviewed by the NSBRI Postdoctoral Fellowship Committee (membership listed below) and scored (0-lowest, 100-highest) based on criteria stated in the RFP. These criteria were (i) scientific merit and program relevance, (ii) research background and qualifications of the candidate, and (iii) research mentor and environment. The scores ranged from 60 to 94, with two applications assessed to be non-responsive/incomplete.

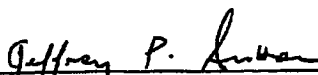
The four highest scoring proposals were selected for two years of funding. The awardees are:

Andrew Judge, Ph.D. Boston University
The Use of Aspirin and Other NSAIDS to Ameliorate Muscle Atrophy Due to Simulated Weightlessness
Mentor: Susan Kandarian, Ph.D.
NSBRI Muscle Alterations and Atrophy Team Self-CRL 5

Luis Cardoso Landa, Ph.D. Mount Sinai School of Medicine
Ultrasonic Assessment of Anisotropic Mechanical Properties of Cancellous Bone after Disuse with and without Anti-resorptive Therapy
Mentor: Mitchell Schaffler, Ph.D.
NSBRI Bone Loss Team Self-CRL 5

Elinor Pulcini, Ph.D. Montana State University-Bozeman
Modeled Microgravity Effects on the Virulence of *Pseudomonas aeruginosa* and *Burkholderia cepacia* in Relation to Spaceflight Crew Health Risks
Mentor: Barry Pyle, Ph.D.
NSBRI Immunology, Infection and Hematology Team Self-CRL 2

Janelle M. Hardisty, Ph.D. The University of Texas Southwestern
The Effect of Gender on Orthostatic Tolerance: A Ground-Based Application of Microgravity Science for Human Cardiovascular Control
Mentor: Benjamin Levine, M.D.
NSBRI Cardiovascular Alterations Team Self-CRL 3



Jeffrey P. Sutton, M.D., Ph.D.
Director, NSBRI

10/1/04

Date

NSBRI Postdoctoral Fellowship Review Committee

Gerald Sonnenfeld, Ph.D. (Chair)
Binghamton University, State University of New York

Jeanne Becker, Ph.D.
NSBRI

Jonathan Clark, M.D.
NASA Johnson Space Center/NSBRI

Roberta Diaz-Brinton, Ph.D.
University of Southern California

Steve Doty, Ph.D.
Hospital for Special Surgery/Cornell

Robert Fitts, Ph.D.
Marquette University

Marlene MacLeish, Ed.D.
Morehouse School of Medicine

Carolyn Randolph, Ph.D.
South Carolina Education Association

Charles Sawin, Ph.D.
NASA Johnson Space Center

Mark Wilson, Ph.D.
Boeing Company

Jeffrey Sutton, M.D., Ph.D. (*Ex Officio*)
NSBRI